



A Systematic Analysis of *Prunus* Species with a Focus on Management Plan of *Prunus africana* (Hook.f.) Kalkman: An Autochthon Plant of Africa

Maninder Karan^{1*}, Ashish Kumar Jena¹, Neetika Sharma¹ and Karan Vasisht¹

¹Faculty of Pharmaceutical Sciences, University Institute of Pharmaceutical Sciences–UGC Centre of Advanced Study, Panjab University, Chandigarh 160014, India.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MK and KV conceptualized and designed the study, supervised the work, analysed the results and monitored the manuscript preparation. Authors AKJ and NS managed the literature searches, collected samples, performed analyses of the study and the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2017/35897

Editor(s):

(1) Thomas Efferth, Chair, Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Germany.

(2) Marcello Iriti, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.

Reviewers:

(1) Dinithi Peiris, University of Sri Jayewardenepura, Sri Lanka.

(2) Divya S. Rajan, Christian College, Kerala University, India.

Complete Peer review History: <http://www.sciencedomain.org/review-history/20771>

Original Research Article

Received 1st August 2017
Accepted 25th August 2017
Published 1st September 2017

ABSTRACT

Aims: To provide complete data on the alike and differentiating characters of the bark of six species of *Prunus* i.e. *P. amygdalus* Stokes, *P. armeniaca* L., *P. cerasoides* Buch.-Ham. ex D. Don, *P. domestica* L. and *P. persica* (L.) Batsch vis-a-vis *P. africana* (Hook.f.) Kalkman of family Rosaceae, with an aim of encouraging the use of more species of *Prunus* against men's problems and be a part of the management plan of *Pygeum*.

Place and Duration of Study: The research work has been carried out in University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India between September 2011 and October 2016.

Methods: Systematic evaluation of the similarity and differences based on the morphological evaluation; anatomical and microscopical description; physico-chemical analysis; total phenolics, flavonoids, flavonols and sterols content; chemical profiling through TLC and HPLC suitably corroborated by chemometric analysis; together with efficacy against oxidative stress and

*Corresponding author: E-mail: maninderkaran@hotmail.com, maninderkaran@pu.ac.in;

inflammation, the important factors involved in disease progression of BPH.

Results: The plants under study showed many comparable features related to morphology, anatomy, microscopy, total content of various active constituents and significant biological activity. The chemical similarity between the species was supported by chemometric analysis of their HPLC fingerprint profile. The remarkable membrane stabilising activity in controlling inflammation and protective effect against oxidative stress corroborated their usefulness in BPH.

Conclusions: The study shall be of interest to people across the globe with diverse backgrounds for making correct identification and suitable selection of the *Prunus* species. It will not only enhance the therapeutic value of more species of *Prunus*, but will directly or indirectly contribute in the preservation of an autochthon plant *P. africana* from the dangers of getting extinct.

Keywords: *Pygeum*; *Prunus* species; comparative study; fingerprint profile; similarity analysis; chemometrics; membrane-stabilizing and antioxidant activity.

1. INTRODUCTION

The tradition of using medicinal plants as a key source of medicines has existed since antediluvian times. Medicinal plants are not only an innate component of various traditional medicine systems, but continue to be the power house of modern drug discovery programs as well. Nearly 35,000 to 70,000 flowering species of an estimated 3.5 million plants have been used medicinally around the world [1]. The number of plant drugs originating directly or indirectly from the natural sources constitutes more than 35% of all drugs and their clear dominance to an extent of 70% or more in some of the disease areas like cancer and infectious diseases is well known [2]. However, a large population in the developing and under-developed countries have a preference for their own systems of indigenous medicine for one or another reason. This aspect has been extensively exploited by big industrial houses more for their own benefits and making the use of traditional medicines a serious issue for biodiversity, especially for those valuable plants which are habitat specific and collected indiscriminately from the wild sources. *P. Africana* (Hook.f.) Kalkman (*Pygeum*), family: Rosaceae is one such plant used extensively for its role in the treatment of benign prostate hyperplasia (BPH) [3,4], but is currently struggling for its survival. It is a popular traditional remedy for treating men's urological diseases in African continent [5,6]. Europe noticed the usefulness of this plant in the 17th century in controlling bladder discomfort and the old men diseases [6,7,8]. The efficacy and safety of *Pygeum* against BPH has been proved through many clinical trials [9,10,11]. The *Pygeum* bark is said to be used not only by the traditional healers but also by local people for BPH by making their own collections [5]. An

important and critical issue with this plant is its inclusion in Appendix II of the CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) owing to over exploitation [3,12,13] and its international trade is regulated [14]. The destructive harvesting through wild collections has led to deforestations leaving little scope for the unusual ability of the plant to regenerate its bark [13,15]. The habitat specific and slow maturity of the plant has further threatened the survival of *P. africana* [15] demanding attention of the scientific community to find possible solutions for the conservation of the species. A strong recommendation by UNESCO to look for the alternative sources, together with Food and Agricultural Organization (FAO) statement that, approaches for the conservation of the species must be kept flexible and diverse focussing on different options [13] made us to plan our study with an exigent need to look for a solution to *P. africana* problem. It aimed at effective utilization of resources widely available across the globe of both wild-collected and cultivated *Prunus* species other than *P. africana*. The work has already started in the direction of using other renewable sources with reports on improved process for *Pygeum* extraction using stem cuttings/twigs of *P. domestica* L. [16,17,18]. *Prunus* is a large genus of around 430 species including a sizeable number of plants of important edible stone fruits well known for their high nutritional value (almonds, cherries, plums, peaches and apricots). The selected plants are not as habitat specific as *Pygeum* and are known to be more widely distributed across the globe as shown in Fig. 1 and Table 1.

Pygeum is a famous remedy for treating benign prostate hyperplasia. All the selected plants viz. *P. amygdalus* Stokes (Almond, Sweet almond and Badam), *P. armeniaca* L. (Apricot, Khurmani

and Zardalu), *P. cerasoides* Buch.-Ham. ex D. Don (Sour cherry, Padam and Paija), *P. domestica* L. (Wild plum, Alubukhara and Alucha) and *P. persica* (L.) Stokes (Peach, Nectarine, Shaftalu and Aru) and the official drug *P. africana* (African cherry, *Pygeum*) are known to be used against inflammation [3,6,7,43,44,45,46] which is one of three major factors involved in BPH [47]. A well-established correlation between BPH and inflammation is reported in literature [48,49]. A careful literature search showed that there is no published report drawing a total comparison between the selected species of *Prunus*. Hence, the present study was planned to provide a comprehensive data on the pharmacognostic, phytochemical and biological comparison of the bark of selected plants covering morphological evaluation, anatomical and microscopical description (transverse section and powder microscopy), physico-chemical analysis and chromatographic fingerprinting. The total phenolics, flavonoids, flavonols and sterols considered responsible for the biological activity were also estimated. HPLC fingerprinting using chemometrics as a statistical tool of similarity analysis and the biological activity principally based on membrane-stabilizing activity for anti-inflammatory evaluation together with antioxidant activity, two of the three key factors involved in BPH were included in the study to strengthen the comparison. Recently, we have reported the anti

BPH activity of the *Prunus* species selected for current study [50]. A preparation (Sitoprin, CR002) based on *P. domestica* (and not *P. africana*) under the name of *Pygeum* [16,17,18] has already come up. Thus, in light of the fact that there is every possibility of the use of *Prunus* species as *Pygeum* 'other than *P. africana*', it is of high relevance to fully characterize the bark of these plants for correct identification and suitable comparison. The strong explanatory notes covering multiple aspects in this study are well timed and will encourage the use of widely available *Prunus* species while helping many countries in utilizing their own resources and contribute in the preservation of an autochthon plant *P. africana* from the dangers of getting extinct.

2. MATERIALS AND METHODS

2.1 Geographical Distribution

The information on the pattern of the distribution of plants under investigation was collected from various resources including Flora, published reports, country databases, web searches. The collected data is compiled both in a map (Fig. 1) as well as in tabulated form (Table 1) for better understanding and the consulted references are cited in table.



Fig. 1. Map showing geographical distribution of the selected species of *Prunus*

2.2 Collection and Authentication of Plants

The collections of the bark of *P. amygdalus*, *P. armeniaca*, *P. cerasoides* and *P. persica* were made in 2010-2011 from Dr. Y.S. Parmar University of Horticulture & Forestry, Nauni, Solan (Himachal Pradesh, India) and that of *P. domestica* from Kangra and its surrounding areas (Himachal Pradesh, India) in 2012. Chemical Resources, Panchkula, India provided the genuine sample of *P. africana* bark. The samples (voucher numbers 236-241 for *P. domestica*, *P. amygdalus*, *P. armeniaca*, *P. cerasoides*, *P. persica* and *P. africana*) were authenticated by National Institute of Science Communication and Information Resources (NISCAIR), New Delhi vide Ref-No NISCAIR/RHMD/Consult/-2012-13/2031/39.

2.3 Chemicals and Reagents

All the chemicals, reagents and solvents used in the study were of analytical grade and purchased from E. Merck Pvt. Ltd., India. β -Sitosterol, ascorbic acid and trolox were obtained from Sigma-Aldrich Chemicals Pvt. Ltd. A reference sample of docosyl ferulate was provided by the Medicinal Chemistry Unit of the Division of Pharmaceutical Chemistry, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India.

2.4 Morphological Evaluation

The organoleptic characters were evaluated by means of the organ of sense and appearance of the drug. The external morphological characters of the bark of all the selected species were noted for the study.

2.5 Anatomical Study and Powdered Microscopy

The transverse section (TS) of the bark of different species of *Prunus* was taken for the anatomical study. The sections were mounted with glycerine and observed under microscope. For powder microscopy, the finely powdered sample was mounted in chloral hydrate and glycerine, covered with a cover slip and examined under microscope. The different cell components (cork, collenchyma, cortex, phloem fibres, sclereids and calcium oxalate crystals) were noted and photomicrographs were taken by using a digital camera fitted with the Nikon-90i

microscope. The powdered samples were also subjected to chemomicroscopy for starch, lignified cells, tannins and anthraquinone derivatives.

2.6 Phytochemical Screening

Reported recently by our laboratory [50].

2.7 Physico-chemical Analysis

Physico-chemical parameters of the powdered drug such as total ash, acid-insoluble ash, water-soluble ash, water-soluble extractive, alcohol-soluble extractive and moisture content were determined according to the standard methods [51].

2.8 Estimation of Total Amount of Phenolics, Flavonoids, Flavonols and Sterols

The total phenolics, flavonoids, flavonols and sterols of each *Prunus* species were determined according to the reported methods [52,53,54,55,56].

2.9 Total Phenolics

The total phenolic content was estimated by the Folin-Ciocalteu colorimetric method, using gallic acid as a standard phenolic compound by slight modification [52]. Briefly, 0.5 mL (in triplicate) of the filtered extract was mixed with 2.25 mL of distilled water and 1.25 mL of 0.2 N Folin-Ciocalteu reagent. After 5 min, 1 mL of the saturated sodium carbonate solution (7.5%) was added. The absorbance of the resulting blue-coloured solution was measured at 765 nm after incubation at 30°C for 1.5 h with intermittent shaking. Quantitative measurements were performed, based on a standard calibration curve of six points: 3.125, 6.25, 12.5, 25, 50 and 100 μ g/mL of gallic acid in methanol. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g of dry material.

2.10 Total Flavonoids

The flavonoids content was determined by the standard procedure using rutin as reference drug [53,54]. Briefly, 1 mL of the plant extract (10 mg/mL) was mixed with 1 mL of aluminium trichloride (20 mg/mL) in methanol and a drop of acetic acid and the volume was made up to 25 mL with methanol. The absorption was measured

at 415 nm after 40 min. Blank samples were prepared from 1 mL of the plant extract and a drop of acetic acid, and then diluted to 25 mL with methanol. The absorption of the standard rutin solution (0.5 mg/mL) in methanol was measured under the same conditions. The experiment was performed in triplicate. The amount of total flavonoids in plant samples expressed as rutin equivalent (RE), was calculated by the following formula:

$$X=(A \cdot m_0)/(A_0 \cdot m)$$

Where X=flavonoid content, mg/mg plant extract in RE; A=absorption of plant extract solution; A₀=absorbance of standard rutin solution; m=weight of plant extract in mg; m₀ = weight of rutin in mg.

2.11 Total Flavonols

The content of flavonols was determined using rutin as a reference standard [53,54]. Briefly, 1 mL of the methanolic drug extract (10 mg/mL) was mixed with 1 mL of aluminium trichloride (20 mg/mL) and 3 mL of sodium acetate (50 mg/mL), kept for 2.5 h and absorbance was read at 440 nm. The absorption of the standard rutin solution (0.5 mg/mL) in methanol was measured under the same conditions. The experiment was carried out in triplicate. The amount of total flavonols in plant samples expressed as rutin equivalent (RE), was calculated by the following formula:

$$X=(A \cdot m_0)/(A_0 \cdot m)$$

Where X=flavonoid content, mg/mg plant extract in RE; A=absorption of plant extract solution; A₀=absorbance of standard rutin solution; m=weight of plant extract in mg; m₀=weight of rutin in mg.

2.12 Total Sterols

The total phytosterols content was determined by Liebermann-Burchard (LB) reagent method [55,56]. The reagent was prepared by taking 50 mL of acetic anhydride in amber glass vial, kept in ice bath for 30 min and then 5 mL of sulphuric acid was added carefully. About 1 g of the plant drug was extracted with 50 mL of chloroform, dried and reconstituted with chloroform up to 8 mL. Next, 1 mL of the drug solution was mixed with 1 mL of prepared LB reagent, the volume was made up to 5 mL with chloroform and the absorption was measured at 625 nm after 5 min. Chloroform was used as blank. The absorbance

of standard β -sitosterol (1 mg/mL) in chloroform was measured using same procedure and conditions. The total phytosterol content (TPC) was calculated as β -sitosterol (g%) using the photometric standard equation:

$$TPC=C_s \cdot A_a/A_s$$

Where: C_s=Standard concentration; A_a=Absorbance of the sample; A_s=Absorbance of the standard.

2.13 Chromatographic Studies

2.13.1 TLC fingerprinting

The comparative TLC chromatograms and total fingerprint profiles with optimum resolution were developed using pre-coated silica gel F₂₅₄ plates [E. Merck (India) Ltd., alumina base, 0.2 mm thickness] and using the solvent systems of toluene:ethyl acetate (8:2) for β -sitosterol and hexane:acetone:formic acid (8:1.5:0.5) for docosyl ferulate. The reference USP markers and extracts were applied as bands of 1 cm width using Camag Linomat 5. The running distance was kept at 8 cm and anisaldehyde-sulphuric acid reagent was used as derivatizing agent followed by heating at 110 °C for 5 min or till the bands developed colour. The TLC fingerprint profiles were recorded as images under UV at 366 nm for docosyl ferulate and under white light after derivatization for β -sitosterol on Camag Reprostar fitted with D×A 252 16 mm camera. Further, the developed TLC plates were scanned at 545 nm (for β -sitosterol) and at 323 nm (for docosyl ferulate) in CAMAG TLC scanner 3. The TLC fingerprint chromatograms were recorded for comparison and an overlay of the densitometric scan of *Prunus* species with docosyl ferulate and β -sitosterol was obtained.

2.13.2 HPLC fingerprinting

The HPLC fingerprint profile of different species of *Prunus* was developed using Aquity HPLC system (Waters Corporation, Milford, MA, USA) equipped with tunable UV detector. The chromatographic separation was performed on Hibar C₁₈ column (E. Merck, 250 × 4.6 mm, 5 μ m) maintained at 30°C, with the detection wavelength set at 323 nm. The mobile phase consisted of water:methanol:acetonitrile (1:91:8) with a flow rate of 1 mL/min for 30 min. The Empower 2 chromatography software was applied in data acquisition and data processing.

2.13.3 Analysis of chemical variability

The HPLC fingerprint profiles of *Prunus* species were generated on a reverse phase column and this data was used for chemometric analysis using SPSS 16.0 software. Hierarchical clustering analysis (HCA) was done using Ward's method and squared Euclidean distance was calculated. Dendrogram was generated based on the peak areas of different peaks obtained in the HPLC fingerprint of different species of *Prunus* to know about the chemical variability.

2.14 Pharmacological Studies

2.14.1 Experimental animals

The animal studies were approved by the Institutional Animal Ethics Committee (IAEC) vide ref. no 282/IEAC dated 30.08.2012 and the animals were used according to the CPCSEA (Committee for the Purpose of Control and Supervision of Experimentation on Animals) guidelines. The male Wistar rats were procured from the Central Animal House, Panjab University, Chandigarh, India. The animals were kept in animal house maintained under normal controlled environment conditions and used for the collection of blood sample.

2.14.2 Membrane stabilisation activity

The rat red blood cell (RRBC) membrane stabilisation method is a simple, economical and reproducible technique used for the estimation of anti-inflammatory activity [57,58]. The inhibition of erythrocyte membrane lysis, thereby stabilizing the RBC membrane is the basis of this test. The experiment was carried out according to the reported procedure reported [59]. The blood was collected under ether anaesthesia from the eye of the rats using aseptic precautions and mixed with equal volume of sterilized Alsever solution.

The blood was centrifuged at 3000 rpm for 10 min. The packed cells were washed thrice with isotonic phosphate buffered solution (pH 7.0) and 10% v/v suspension was made with isotonic salt solution. The standard (diclofenac sodium) and test samples were prepared by suspending the residues in 0.32% hypotonic solution in PBS (pH 7.0). Briefly, the assay mixture contained 0.5 mL of RRBC suspension and 4.5 mL of the hypotonic solution containing either extract or standard. The control was taken without any test substance. All the assay mixtures were

incubated at room temperature for 30 min and centrifuged at 3000 rpm for 15 min. The haemoglobin content in the supernatant solution indicating the extent of haemolysis was estimated using spectrophotometer at 560 nm.

$$\text{Percentage protection} = \frac{[(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}})]}{\text{OD}_{\text{control}}} \times 100$$

2.14.3 Antioxidant activity

2.14.3.1 DPPH[·] assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity was determined by slightly modified spectrophotometric method [60]. A solution of DPPH radical in methanol (100 µM) was prepared freshly and to measure the scavenging activity, 1 mL aliquot of this solution was mixed with 1 mL of the test samples and standard ascorbic acid at different concentrations and the volume was made up to 4 mL with methanol. The methanolic solution of DPPH was used as control. After shaking, the test tube containing solutions were incubated for 30 min at room temperature and kept away from light. The absorbance was measured at 517 nm, the percentage of DPPH radical-scavenging activity of each test drug was calculated and the results were expressed as IC₅₀ value. All the determinations were performed in triplicate.

$$\text{Per cent scavenging activity} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{test}})}{\text{Abs}_{\text{control}}} \times 100$$

2.14.3.2 ABTS assay

The ABTS radical-scavenging activity was performed following the reported method [61]. The ABTS radical cation (ABTS^{·+}) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulphate and the mixture was allowed to stand in the dark for 12-16 h at room temperature to produce a dark coloured solution. This stock solution was diluted with methanol to give a final absorbance value of about 0.7 ± 0.02 at 734 nm before use. Different concentrations of the test substance were prepared and about 1 mL of the sample solution was mixed with 1 mL of the diluted working ABTS^{·+} solution, and volume was made up to 4 mL with methanol. The absorbance was measured 6 min after the initial mixing at 734 nm. The absorbance of the control sample was measured using 1 mL of the ABTS solution with 3 mL of methanol. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), the water

soluble analogue of vitamin E was used as a reference standard. The standard curve was prepared by measuring the reduction of the ABTS⁺ solution with different concentrations of trolox solution exactly after 6 min with initial mixing. The percentage inhibition was calculated according to the formula and expressed as IC₅₀ value. Further, the radical-scavenging activity was expressed as the trolox equivalent antioxidant capacity (TEAC).

$$\text{Per cent scavenging activity} = 100 - \left(100 \times \frac{\text{Abs}_{\text{test}} - \text{Abs}_{\text{blank}}}{\text{Abs}_{\text{control}}} \right)$$

2.15 Statistical Analysis

Statistical analysis was done using one way analysis of variance followed by Dunnett's *t* test. The values *P* < 0.05 were considered significantly.

3. RESULTS

3.1 Geographical Distribution

The selected plants under study were found to be growing very widely all over the globe. Their distribution with country details is given in Table 1 and Fig. 1.

3.2 Morphological Description

The organoleptic characters noted for the *Prunus* species under study showed that the stem bark occurs as flat or curved pieces; varying in length and width; outer surface rough, uneven, wrinkled, having exfoliating cork and blackish-brown or yellowish-brown in colour with fibrous/rough/laminated fracture. The inner surface is largely uneven, brown to buff in colour with splintery/fibrous fracture. The taste is bitter and having a characteristic odour. The observed characteristic organoleptic features of bark of *Prunus* species (Fig. 2A-F) are listed in Table 2.

3.3 Microscopic Evaluation

3.3.1 Anatomical description

The microscopic structures of all the samples were studied by observing hand-cut transverse sections at 200X magnification using Nikon-90i microscope. Grossly, the TS of bark showed typical pattern constituting the cork, collenchyma, cortex, phloem, phloem fibres, medullary rays, sclereids and calcium oxalate crystals. The cork consisted of well-developed compact zone of cells, exfoliating at many places, occasionally

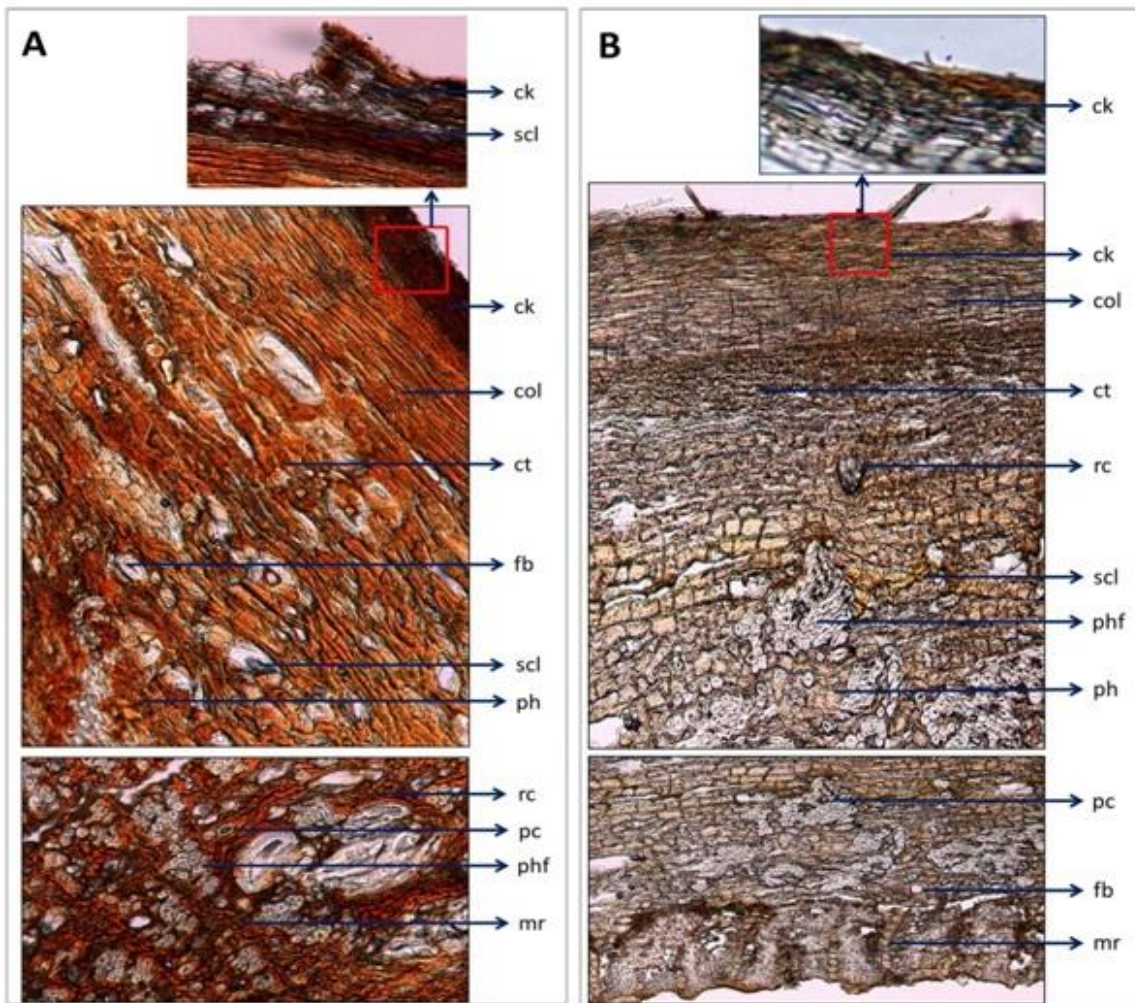
traversed with sclereids as a continuous layer or in patches lying next to it. The thickest zone of cork tissue was observed in *P. cerasoides* (40-50 layers) and thinnest in *P. armeniaca* (8-10 layers). The isolated sclereids were also present, scattered within cortex and phloem. The cork is followed by layers of tangentially elongated cells of collenchyma. The multi-layered cortex constituting an oval to rounded parenchyma cells was characterized by the presence of phloem fibres which occur singly and also in groups. The widest zone of cortex was observed in *P. amygdalus* (60-70 layers) and that of *P. cerasoides* was minimum in width (20-25 layers). The phloem consisted of phloem parenchyma, phloem fibres, traversed by the wavy, uni- or bi- to multiseriate medullary rays and occasionally by calcium oxalate crystals and sclereids. The medullary rays were uniseriate in *P. amygdalus*, multiseriate in *P. africana* and uni- to multiseriate in other four species. The calcium oxalate crystals of rosette & prismatic shape and of various sizes were seen throughout the parenchymatous cells of cortex and also found scattered in the phloem region. The images of representative transverse sections of these drugs are shown in (Fig. 3A-F) and the characteristic details have been summarized in Table 3.

3.3.2 Powder microscopy

The powder microscopy showed the fragments of polygonal to rectangular thick walled cork cells embedded with dark brown tannin content. Thick-walled, both lignified and non-lignified fibres having narrow lumen with pointed to bluntly pointed ends, except for *P. africana* which consisted of lignified fibres only were observed. The size of fibres was largest (upto 95 µm) in *P. africana* and *P. persica* and smallest (upto 13 µm) in *P. amygdalus*. The elongated and/or isodiametric sclereids with wide or narrow-lumen were present in all the species. Fragments of vessels with scalariform and/or spiral thickenings were observed in *P. africana*, *P. amygdalus*, *P. armeniaca* and *P. persica*. Plenty of large and variable sized prismatic (upto 9 µm) and/or rosette (upto 10 µm) shaped crystals scattered as such throughout or embedded in the parenchymatous cells were seen which were few in number in *P. africana*, *P. amygdalus* and *P. persica*. The chemomicroscopy study showed the presence of lignin, tannins and anthraquinone derivatives in all the samples. The detailed distinguishing microscopic features of each bark sample are tabulated in Table 4 and Fig. 4A-H.



Fig. 2. Morphological characteristics of the bark of different species of *Prunus*: (A) *P. africana*; (B) *P. amygdalus*; (C) *P. armeniaca*; (D) *P. cerasoides*; (E) *P. domestica* and (F) *P. Persica*



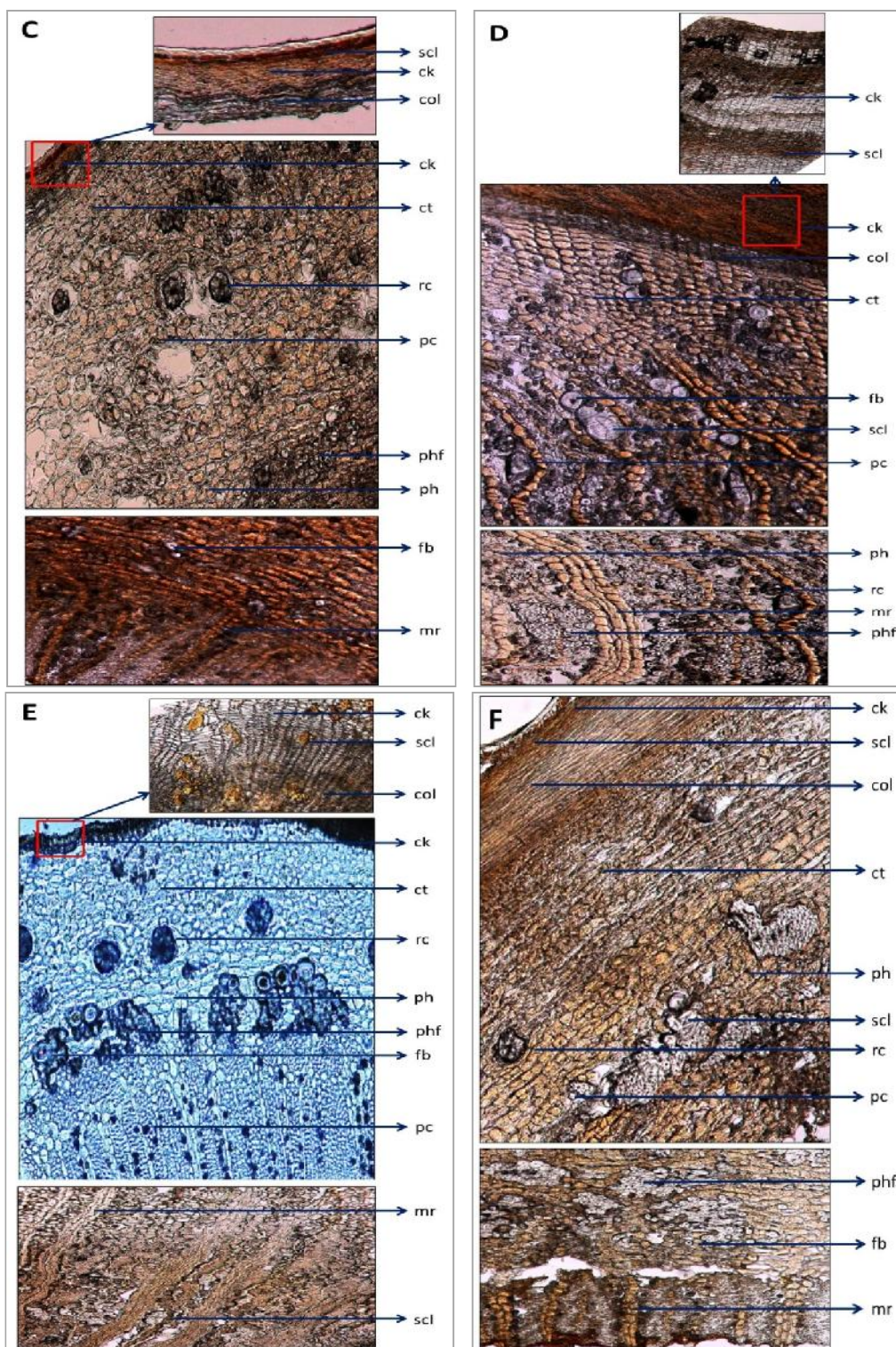


Fig. 3. Transverse section of the bark of different species of *Prunus* x10; (A) *P. africana*; (B) *P. amygdalus*; (C) *P. armeniaca*; (D) *P. cerasoides*; (E) *P. domestica*; (F) *P. persica*; ck: cork; scl: sclereids; col: collenchyma; ct: cortex; ph: phloem; fb: fibre; rc: rosette crystal; pc: prismatic crystal; phf: phloem fibre; mr: medullary rays

Table 1. Summary of the selected species of genus *Prunus*

Name of the species	Common name	Distribution	Reported constituents of the bark	Pharmacological activity of the bark	Reference(s)
<i>P. africana</i> (Hook. f.) Kalkman	African plum; red stinkwood	Africa (Cameroon, Nigeria, Ethiopia, Madagascar, Burundi, Democratic Republic of Congo, Rwanda, Tanzania and Uganda, Kenya, Sudan, Tanzania and Uganda, Malawi, Tanzania and Zambia, Mozambique and Zimbabwe, Lesotho, South Africa and Swaziland)	Myristic acid, palmitic acid, β -sitosterol, campesterol, daucosterol, ursolic acid, oleanolic acid, friedelin, epimaslinic acid, <i>n</i> -tetracosanol, <i>n</i> -docosanol, docosyl ferulate and amygdalin	Remedy for stomach-ache, constipation, toothache, cough, fever, muscle pains, weak joints, general body pains and BPH.	[3,19,20,21,22,23,24,25, 26,27,28,29]
<i>P. amygdalus</i> Batsch	Almond; bitter almond; sweet almond	India, Arabian Peninsula, Western Asia, Mediterranean region, temperate Asia, Australia and USA (California, Alabama and Utah)	Kaempferol, quercetin 3,4'-diglycoside and persicogenin 3'-glucoside	NR	[19,20,21,22,23,30,31,32]
<i>P. armeniaca</i> L.	Common apricot	India, China, Mid-Asia, widely cultivated, Russia, Europe (Germany and Turkey), Mongolia, Australia and USA	NR	NR	[19,22,31,33,34]
<i>P. cerasoides</i> D. Don	Sour cherry; wild Himalayan cherry; padam (India)	China, Eastern Asia, Indian Subcontinent, Indo-China, Bhutan, Thailand, Vietnam, Myanmar	Sakuranetin, naringenin 4'-methyl ether xyloside, taxifolin, β -sitosterol, chrysophanol, emodin, physcion and orientalone	In skeleto-muscular problems, sprain/fracture, hair oil massage and used in neuralgia.	[19,20,21,22,23,31,32,35, 36,37]
<i>P. domestica</i> L.	European plum and common plum	Widely cultivated, probable origin Eurasia (China, India, Indonesia, Pakistan, Russia, Germany, Iran, Turkey, Thailand, UK, France, Italy, South Korea, Spain, Ukraine, Malaysia, Greece), Australia, Costa Rica, Guatemala, Honduras, Mexico, Canada, Mexico, Peru, Denmark and USA	NR	Febrifuge	[19,20,21,22,23,32,38,39, 40]
<i>P. persica</i> (L.) Batsch	Peach, nectarine, aru and alu	India and China, Australia, Canada, Peru, Argentina and USA	Eriodictyol, persicogenin, persiconin, β -sitosterol	Leprosy, jaundice, antibacterial and insecticidal	[19,20,21,22,23,24,32,40, 41,42]

NR: Not reported

Table 2. Morphological characters of the bark of different species of *Prunus*

Feature	<i>P. africana</i> (Fig. 2A)	<i>P. amygdalus</i> (Fig. 2B)	<i>P. armeniaca</i> (Fig. 2C)	<i>P. cerasoides</i> (Fig. 2D)	<i>P. domestica</i> (Fig. 2E)	<i>P. persica</i> (Fig. 2F)	
Colour	Outer surface Inner surface	Brown Brown	Blackish-brown Buff	Dark-brown Buff	Greyish -brown Buff	Dark-brown Buff	Yellowish-brown Buff
Length (cm)		4-7	5-15	10-15	10-15	5-15	10-25
Breadth (cm)		2.5-3.5	0.7-1.0	0.5-0.8	2.0-4.0	2.5-3.5	0.8-1.2
Thickness (cm)		0.8-1.0	0.2-0.3	0.1-0.2	0.1-0.3	0.1-0.2	0.1-0.2
Texture		Flat, dense, outer surface wrinkled, exfoliated, inner surface having longitudinal striations	Curved, outer surface wrinkled, exfoliated, inner surface uneven	Curved, outer surface highly wrinkled, exfoliated, inner has longitudinal striations	Flattened, external surface showed transverse wrinkles, inner surface uneven	Curved, outer surface wrinkled, exfoliated, inner surface having longitudinal striations	Flattened, outer surface wrinkled, exfoliated, shows lenticels and inner surface uneven
Fracture	Outer surface Inner surface	Fibrous Splintery	Rough Splintery	Short Splintery	Rough Fibrous	Rough Fibrous	Laminated Fibrous
Odour		Characteristic	Unpleasant	Pleasant	Unpleasant	Pleasant	Very faint
Taste		Bitter	Bitter	Not characteristic	Mild bitter	Mild bitter	Not characteristic

Table 3. Anatomical characters of the bark of different species of *Prunus*

Feature	<i>P. africana</i> (Fig. 3A)	<i>P. amygdalus</i> (Fig. 3B)	<i>P. armeniaca</i> (Fig. 3C)	<i>P. cerasoides</i> (Fig. 3D)	<i>P. domestica</i> (Fig. 3E)	<i>P. persica</i> (Fig. 3F)
Cork	Brownish thick-walled, compact and exfoliated (10-15 layers)	Brownish thick-walled, compact and exfoliated (10-12 layers)	Brownish thick-walled, compact and exfoliated (8-10 layers)	Brownish thick-walled and compact (40-50 layers)	Brownish thick-walled, compact and exfoliated (25-30 layers)	Brownish thick-walled, compact and exfoliated (10-15 layers)
Collenchyma	Tangentially elongated cells (15-20 layers)	Tangentially elongated cells (10-15 layers)	Tangentially elongated cells (5-7 layers)	Tangentially elongated cells (8-10 layers)	Tangentially elongated cells (10-15 layers)	Tangentially elongated cells (15-20 layers)
Cortex	Oval to rounded parenchymatous (40-50 layers)	Oval to rounded parenchymatous (60-70 layers)	Oval to rounded parenchymatous (50-60 layers)	Oval to rounded parenchymatous (20-25 layers)	Oval to rounded parenchymatous (40-50 layers)	Oval to rounded parenchymatous (20-30 layers)
Medullary rays	Multiseriate	Uniseriate	Uniseriate & biseriate	Uniseriate to multiseriate	Uniseriate to multiseriate	Uniseriate to multiseriate
Phloem fibre	Abundant, single and in groups, present in cortex & in between medullary rays	Few, single and in groups, present in small and large groups in cortex and in between medullary rays	Few, single and in groups, small groups especially in cortex and in between medullary rays	Abundant, single, small and large groups in cortex & in between medullary rays	Abundant, single and in small and large groups in cortex & in between medullary rays	Few, single and in groups present in small and large groups in cortex & in between medullary rays
Sclereids	Aa a continuous layer next to cork; also present singly scattered within cortex and phloem	Present singly or in groups, scattered within cortex and phloem	Aa a continuous layer next to cork	Aa a continuous layer next to cork; also present singly, scattered within cortex and phloem	Present singly, scattered next to cork and in phloem	Aa a continuous layer next to cork

Feature	<i>P. africana</i> (Fig. 3A)	<i>P. amygdalus</i> (Fig. 3B)	<i>P. armeniaca</i> (Fig. 3C)	<i>P. cerasoides</i> (Fig. 3D)	<i>P. domestica</i> (Fig. 3E)	<i>P. persica</i> (Fig. 3F)
Crystals	Few, scattered, both rosette & prismatic present in cortex and phloem region	Few, scattered, both rosette & prismatic and prismatic present in groups in cortex and phloem region	Abundant, scattered, both rosette & prismatic and rosette present in groups in cortex and phloem region	Abundant, scattered, in groups, both rosette & prismatic present in cork, cortex and phloem region	Abundant, scattered, both rosette & prismatic and prismatic present in groups in cortex and phloem region	Few, scattered, both rosette & prismatic present in cortex and phloem region

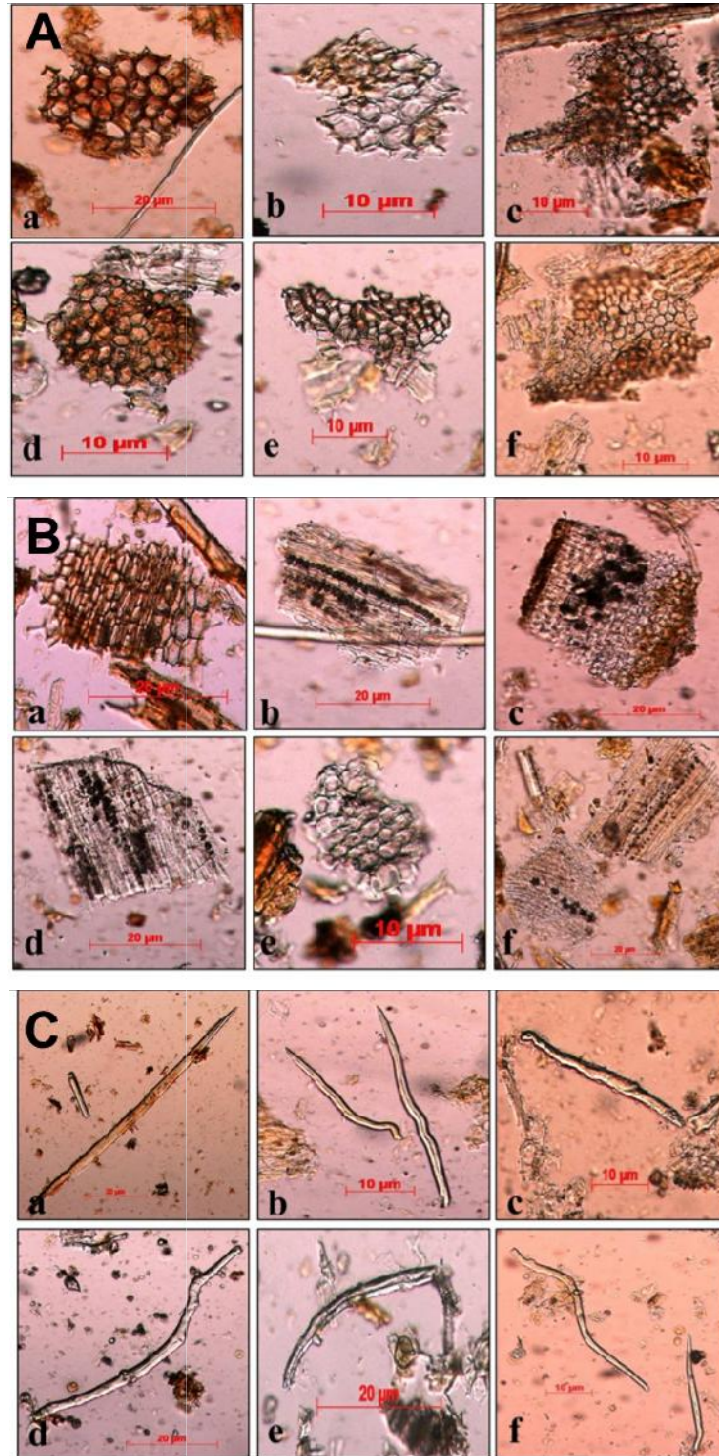
Table 4. Powder microscopy characteristics of the bark of different species of *Prunus*

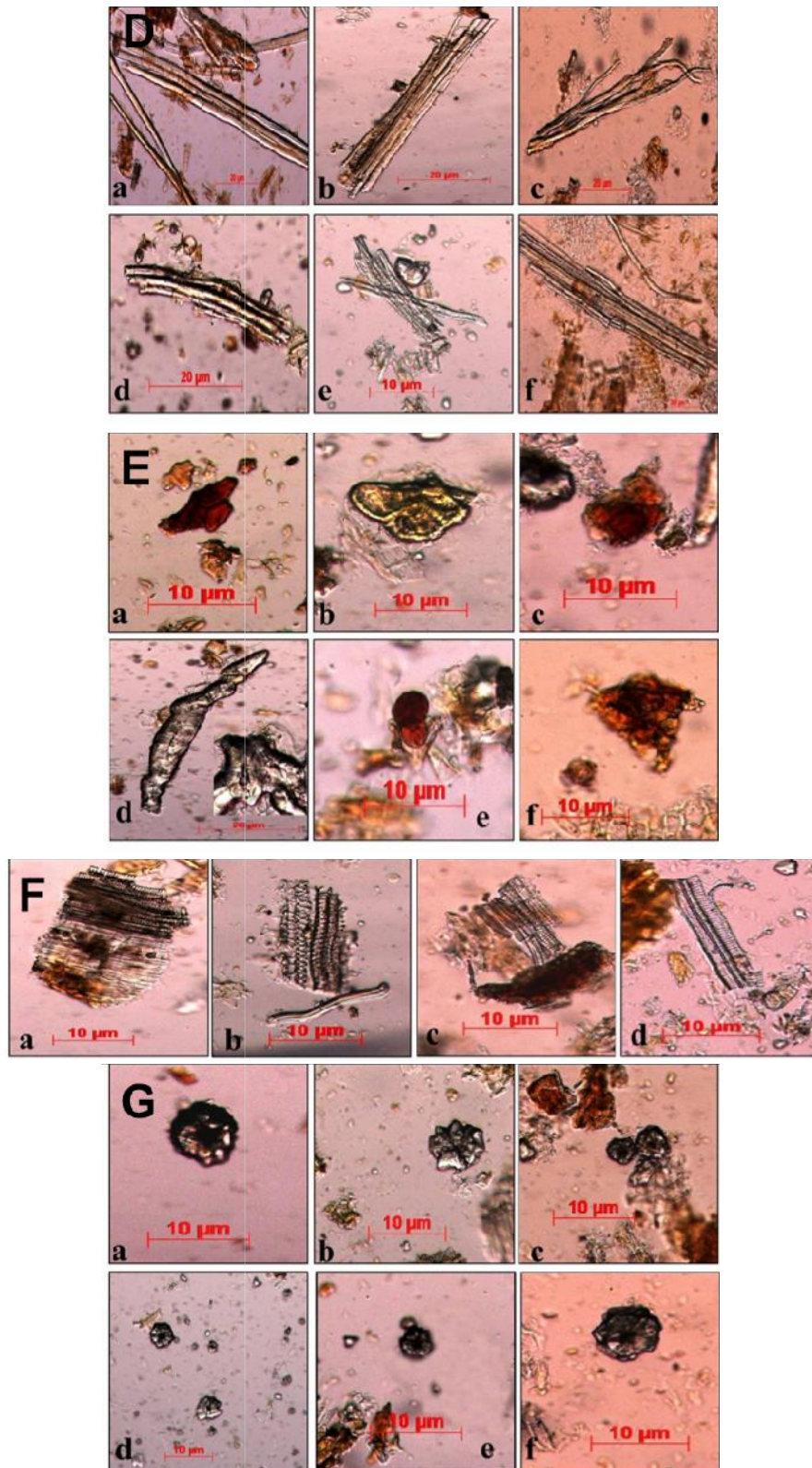
Feature	<i>P. africana</i>	<i>P. amygdalus</i>	<i>P. armeniaca</i>	<i>P. cerasoides</i>	<i>P. domestica</i>	<i>P. persica</i>
Cork (Fig. 4A)	Tangentially thick walled, polygonal to rectangular, embedded with dark brown tannin content	Tangentially thick walled, polygonal to rectangular, embedded with dark brown tannin content	Thick walled, polygonal to rectangular, embedded with dark brown content	Thick walled, polygonal to rectangular, embedded with dark brown tannin content	Tangentially thick walled, polygonal to rectangular, embedded with dark brown tannin content	Thick walled, polygonal to rectangular, embedded with dark brown tannin content
Parenchyma (Fig. 4B)	Rounded, phloem region	Rectangular, parenchyma having sheath of calcium oxalate crystals in cortex region	Rounded, parenchyma having sheath of calcium oxalate crystals in phloem region	Rectangular, parenchyma having sheath of calcium oxalate crystals in cortex region	Rectangular, cortex region	Rectangular, parenchyma having sheath of calcium oxalate crystals in cortex region
Phloem fibre (Fig. 4C and D)	Abundant, single or in groups with indistinct lumen, straight, lignified, few conspicuous pits, pointed ends, length 40-90 µm	Abundant, single or in groups, with narrow distinct lumen, frequently bent, both lignified and non-lignified, inconspicuous pits, pointed ends, length 13-66 µm	Few, single or group of fibres with narrow indistinct lumen, straight, both lignified and non-lignified, inconspicuous pits, bluntly pointed ends, length 30-65 µm	Few, single fibre with narrow distinct lumen, frequently bent, both lignified and non-lignified, inconspicuous pits, bluntly pointed ends, length 22-70 µm	Abundant, single fibre with thick distinct lumen, frequently bent, both lignified and non-lignified, inconspicuous pits, pointed bluntly ends, length 20-90 µm	Abundant, single fibre with narrow distinct lumen, frequently bent, both lignified and non-lignified, inconspicuous pits, both pointed and bluntly pointed ends, length 20-95 µm
Sclereids (Fig. 4E)	Groups of 2-3, circular to oval in shape, wide lumen and contain brown pigment (2.5-8.0 µm)	Groups of 4-5, circular to oval in shape, wide lumen and contain yellowish-brown pigment (3.5-13 µm)	Groups of 2-3, circular to oval in shape, narrow lumen and contain yellowish-brown pigment (1.8-4.5 µm)	Single or in groups of 2-3, elongated or oval shape, narrow lumen. (3.0-5.3 µm)	Groups of 2-3, circular in shape, narrow lumen and contain yellowish-brown pigment (1.6-4.7 µm)	Groups of 3-4, circular to oval in shape, narrow lumen and contain yellowish-brown pigment (1.5-6.5 µm)
Vessels (Fig. 4F)	Scalariform	Scalariform and spiral (helical)	Scalariform	Not observed	Not observed	Scalariform
Calcium oxalate crystals (Fig. 4G and H)	Few, both prismatic (0.4-8.0 µm) and rosette (2.0-8.0 µm)	Few, both prismatic (0.5-5.0 µm) and rosette (0.5-8.0 µm)	Abundant, prismatic (0.3-5.5 µm) and few rosette (1.8-6.0 µm)	Abundant, both prismatic (0.5-9.0 µm) and rosette (0.7-10 µm)	Abundant, both prismatic (0.3-8.5 µm) and rosette (0.8-6.5 µm)	Few prismatic (0.6-7.0 µm) and abundant rosette (0.3-7.5 µm)

3.3.3 Physico-chemical evaluation

The different physico-chemical parameters like loss on drying, total ash, acid-insoluble ash,

water-soluble ash, alcohol-soluble and water-soluble extractive were evaluated for the bark of selected species of *Prunus* and the results are given in Table 5.





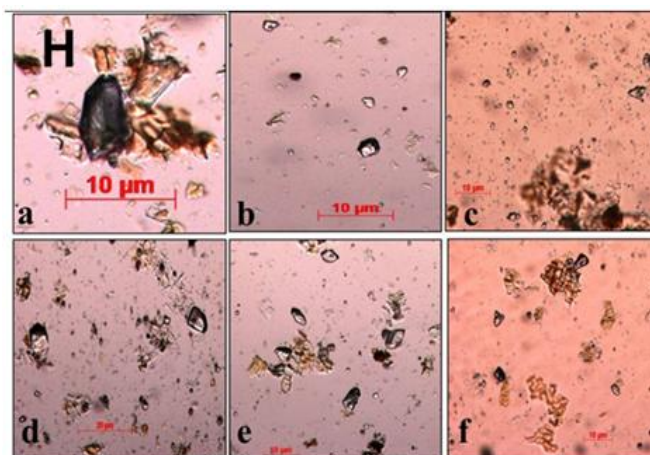


Fig. 4. Powder microscopy of the bark of different species of *Prunus* (A) cork cell, (B) parenchyma containing clusters of calcium oxalate crystals, (C & D) fibre & group of fibre, (E) sclereids, (F) vessels and (G & H) rosette & prismatic calcium oxalate crystal; (a) *P. africana*; (b) *P. amygdalus*; (c) *P. armeniaca*; (d) *P. cerasoides*; (e) *P. domestica* and (f) *P. persica*

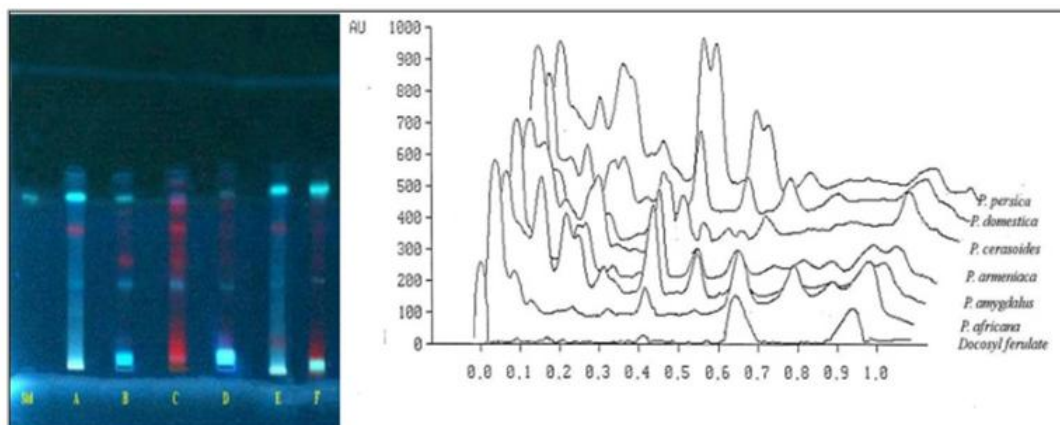


Fig. 5. TLC densitometric scan of bark of different species of *Prunus* using standard docosyl ferulate at UV₃₆₆; (A): *P. africana*; (B): *P. amygdalus*; (C): *P. armeniaca*; (D): *P. cerasoides*; (E): *P. domestica*; (F): *P. persica*

3.3.4 Total phenolics, flavonoids, flavonols and sterols content

The plant phenolics and sterols constitute the major class of compounds and are considered as one of the most diverse and widespread group of natural phytoconstituents. The result of phytochemical screening revealed the presence of phenolics (flavonoids) and sterols. Therefore, their content was determined in all the species of *Prunus*. The total phenolics was measured using Folin-Ciocalteu's (FC) assay method, total flavonoids and its subclass flavonols were estimated using aluminium chloride method,

while the total sterols content was determined by Liebermann-Burchard (LB) reagent method. The content of total phenolics was expressed as gallic acid equivalent (GAE), flavonoids and flavonols were expressed as rutin equivalent (RE), whereas total sterols as β -sitosterol (g%) equivalent. The total content of phenolics and phytosterols was found to be maximum with comparable levels in *P. domestica* and *P. africana*. Both these species also exhibited close values of total flavonoid and flavonol content, though their maximum content was observed in *P. cerasoides* and *P. amygdalus*. Further details have been summarized in Tables 6 and 7.

Table 5. Physico-chemical evaluation of different species of *Prunus*

Sample	Loss on drying (%)	Total ash (%)	Acid-insoluble ash (%)	Water-soluble ash (%)	Alcohol-soluble extractive (%)	Water-soluble extractive (%)
<i>P. africana</i>	0.18	3.8	0.63	1.10	13.2	14.6
<i>P. amygdalus</i>	3.9	4.5	0.11	0.65	18.5	19.2
<i>P. armeniaca</i>	3.7	4.8	0.07	0.62	11.6	17.4
<i>P. cerasoides</i>	2.7	10.5	1.38	3.5	22.2	18.5
<i>P. domestica</i>	3.1	4.3	0.45	1.26	13.3	9.5
<i>P. persica</i>	4.8	4.1	0.08	0.48	19.7	23.7

Table 6. Total flavonoid, flavonol and phytosterol content in different species of *Prunus*

Sample	Total flavonoids (mg rutin equivalents/g plant extract)	Total flavonols (mg rutin equivalents/g plant extract)	Total phytosterol contents (g%)
<i>P. africana</i>	36.4	23.18	6.71
<i>P. amygdalus</i>	47.0	23.21	6.06
<i>P. armeniaca</i>	28.0	14.22	4.56
<i>P. cerasoides</i>	49.82	19.34	2.63
<i>P. domestica</i>	32.8	22.15	6.33
<i>P. persica</i>	35.1	18.87	3.90

Table 7. Total phenolic content and radical-scavenging activity of bark of different species of *Prunus*

Sample	Assays	Total phenols (mg gallic acid equivalents/g plant extract)	DPPH IC ₅₀ (µg/mL)	ABTS	
				IC ₅₀ (µg/mL)	TEAC
<i>P. africana</i>		69.02	6.02	3.8	0.50
<i>P. amygdalus</i>		53.67	14.79	5.85	0.77
<i>P. armeniaca</i>		37.06	15.42	6.19	0.82
<i>P. cerasoides</i>		61.90	8.38	4.22	0.56
<i>P. domestica</i>		71.31	3.89	3.7	0.49
<i>P. persica</i>		59.94	12.64	4.85	0.64
Positive reference		-	19.74	7.58	-

*Positive reference: DPPH- Ascorbic acid; ABTS-Trolox

3.3.5 Chromatographic evaluation

3.3.5.1 TLC studies

The common as well as different bands of TLC fingerprints provided useful information for the easy identification, authentication and comparison of different species of *Prunus* (Figs. 5 and 6). Docosyl ferulate was observed in all samples under UV₃₆₆ at R_f 0.63. The overall profile of *P. domestica* closely matched with *P. africana* (Fig. 5). For β -sitosterol, all the samples showed pink to purple colour band of β -sitosterol at R_f 0.5 under white light. All the spots in *P. africana* visible under white light were seen in *P. domestica*. Other species also showed fairly good similarity with each other (Fig. 6) as well as with *P. africana*.

3.3.5.2 HPLC studies and chemometric analysis

Chemometric analysis is a technique applied to solve both the predictive and descriptive problems in experimental areas such as the plant extracts, food and biological investigations. On the basis of HPLC fingerprint profile of different species of *Prunus* (Fig. 7), the identification of variations was accomplished by hierarchical clustering analysis. A correlation was obtained among the different species based on the area under curve (AUC) of the peaks and retention time (R_t) of the different components present in the plants. The dendrogram obtained (Fig. 8) placed 6 species of *Prunus* into three clusters with *P. africana* and *P. domestica* in one group, *P. amygdalus*, *P. persica*, *P. armeniaca* in the

second group and *P. cerasoides* in the third group. The results clearly indicated a close matching of chemical profile of *P. africana* with *P. domestica*.

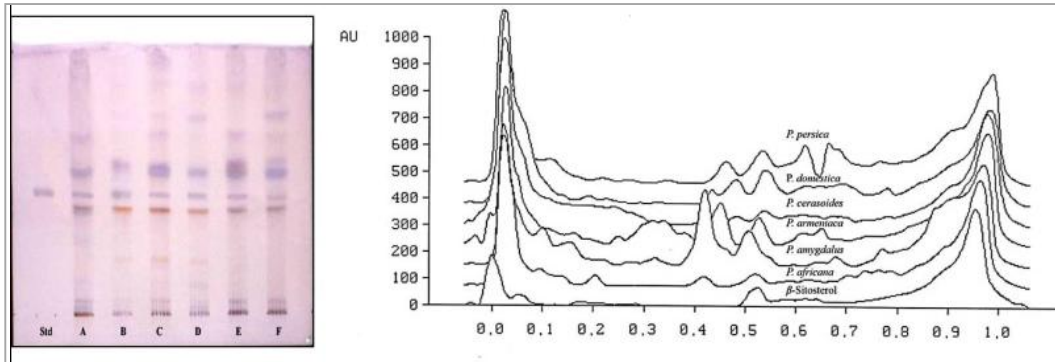


Fig. 6. TLC densitometric scan of bark of different species of *Prunus* using standard β -sitosterol. (A): *P. africana*; (B): *P. amygdalus*; (C): *P. armeniaca*; (D): *P. cerasoides*; (E): *P. domestica* and (F): *P. persica*

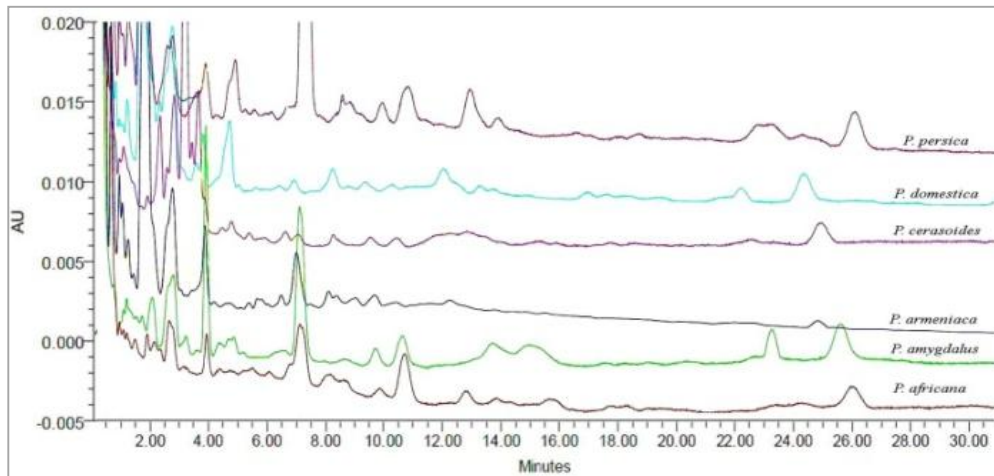


Fig. 7. HPLC fingerprint profile of bark of different species of *Prunus*

Dendrogram using Ward Method

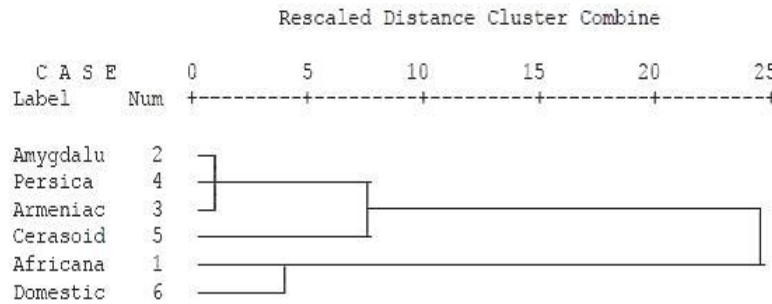


Fig. 8. Dendrogram showing clustering of different species of *Prunus* on the basis of HPLC fingerprint profile

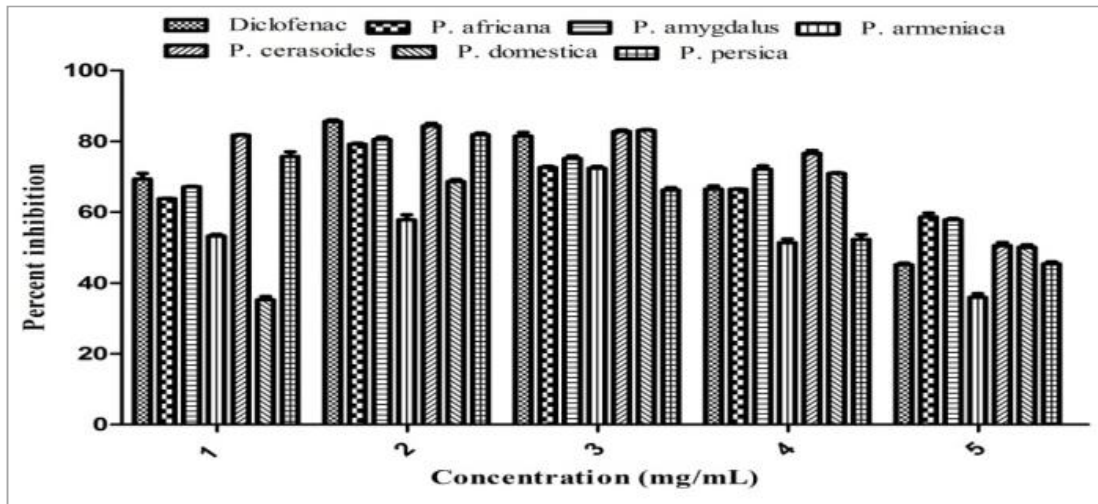


Fig. 9. Membrane stabilizing activity of different species of *Prunus*. The difference with respect to diclofenac group was not significant at $P < 0.05$

3.3.6 Biological studies

3.3.6.1 Membrane stabilizing activity

The *in vitro* RBC membrane stabilizing method was used to investigate the anti-inflammatory activity. During the inflammation process, hydrolytic components and lysosomal enzymes are released from the phagocytes to the extracellular space causing the tissue and organelles damages [62]. It has been seen that the lysis of RBC membrane and haemoglobin takes place on exposure to the hypotonic solution. The stabilization of lysosomal membrane plays a significant role in limiting the inflammatory response by preventing the release of lysosomal constituents, an activated neutrophil which is known to aggravate the tissue inflammation and damage [63]. The anti-inflammatory drugs acts by either stabilizing the lysosomal membrane or inhibiting lysosomal enzymes release. The membrane stabilizing anti-haemolytic effect of the plant extracts by inhibiting hypotonicity induced lysis of the erythrocyte membrane was taken as a measure of the anti-inflammatory activity [58]. All the six *Prunus* species showed significant activity with 72-84% membrane stabilizing effect against 84% shown by diclofenac. Four of the six species (*P. africana*, *P. amygdalus*, *P. cerasoides* and *P. persica*) and diclofenac exhibited dose dependent effect up to second dose level while the other two species (*P. armeniaca* and *P. domestica*) showed the effect up to third dose level beyond which a decreased

effect in per cent inhibition was observed (Fig. 9).

3.3.6.2 Antioxidant activity

The oxidative stress induced prostatic inflammation augments the progression of BPH. The antioxidants have potential free-radical scavenging properties and phenolic compounds are widely distributed natural antioxidants. The *in vivo* antioxidant effect in the prostate tissue and the presence of flavonoids and phenolics has been recently established in all these plants [50]. The antioxidant activity of the methanolic extract of the bark of the selected species of *Prunus* was investigated using the most versatile and widely recognized models of chemical assay.

The *Prunus* species showed highly significant and promising radical scavenging activity that was better than the standard drugs. Among the Indian species, *P. domestica* showed highest activity with IC_{50} value of 3.89 $\mu\text{g/mL}$ as compared to 19.74 $\mu\text{g/mL}$ of ascorbic acid in DPPH assay. Similarly, in ABTS assay the highest radical-scavenging activity was observed in *P. domestica* and *P. africana* with IC_{50} value of 3.7 and 3.8 $\mu\text{g/mL}$, as compared to 7.58 $\mu\text{g/mL}$ of the standard drug trolox. Further, the radical-scavenging activity was expressed as the trolox equivalent antioxidant capacity. The results indicated that the stem bark of *Prunus* species has a higher TEAC than the standard drug. Hence, it is expected that the *Prunus* species under study play a definite role in checking the

formation of ROS and are expected to restore the antioxidant enzymes associated not only with BPH but also in other diseases as well. The results of antioxidant activity are shown in Table 7.

4. DISCUSSION

P. africana (*Pygeum*) is currently the only African species of *Prunus* used as a dietary supplement against benign prostatic hyperplasia and related disorders. To preserve the species on account of over-exploitation, a strong need for alternative sources of African bark is being repeatedly felt and recommended at international level by various organizations including UNESCO. Different strategies for the conservation of *P. africana* have been described from time to time including protection of germ plasm and formulating new regulations [15]. Food and Agriculture Organization of the United Nations (FAO) has recommended that the approaches for the conservation of the species must be kept flexible and diverse focussing on different options [13,15,64,65]. The different possible policies available to conserve the natural resources are: (a) establishment of more conservation areas and cultivation of slow growing plants on large scale, (b) public awareness about the plant diversity and its importance in healthcare systems, (c) use of the alternative species and vegetative renewable plant parts like leaves, young stems and fruits [66]. Hence, the plants which are: edible with high nutritional value; medicinal like *Prunus*; and abundantly available across the globe as wild or cultivated crops, in fact, represent an important focus of research not only to discover active substances with the therapeutic efficacy contained in them, but also provide an opportunity to protect the threatened biodiversity. With this background and in continuation to our recently published report on antiBPH effect of the *Prunus* species, the present study was accomplished with an objective to make a befitting comparison of these species for the benefit of users.

The phytochemical screening had shown the presence of similar type of phytoconstituents in the bark of selected plants including *P. africana*. The macroscopic and microscopic characters essential for the standardization and quality control of medicinal crude drugs were established using gross morphology, transverse section and powder microscopy of the bark. All the drugs showed usual composition of the bark. The most striking feature was the presence,

number and size of fibres, sclereids and calcium oxalate crystals. The fibres occur in abundance in *P. africana*, *P. amygdalus*, *P. domestica* and *P. persica* with size reaching upto 95 μm in *P. persica*. The groups of sclereids were seen in all the species with maximum size reaching upto 53 μm in *P. cerasoides*. Both prismatic and rosette crystals were present in abundance in *P. armeniaca*, *P. cerasoides* and *P. domestica* with size going upto 10 μm in *P. cerasoides*. The maximum content of different ash values and alcoholic soluble extractive value was observed for *P. cerasoides*. The test values for identity, purity and strength were determined for loss on drying, total ash, acid-insoluble ash, water-soluble ash, alcohol and water-soluble extractive. The characteristic features have been elaborated as images in Figs. 2-4 with more details in Tables 2-5.

The USP specifies two bioactive markers, β -sitosterol and docosyl ferulate for ensuring the quality of *P. africana* bark. The presence of two official markers docosyl ferulate and β -sitosterol was recently shown by us in all the investigated species [50]. In light of the fact, that no single component is said to be responsible for activity [9,11], the overlay absorption spectra obtained in the TLC densitometry representing total fingerprint was analysed. Although all the six species showed the presence of β -sitosterol and docosyl ferulate but the overall chemical profile of *P. africana* and *P. domestica*, after derivatization and under UV was matching more closely. The total sterol content was further estimated and expressed as β -sitosterol equivalent (g%). The sterol content in *P. africana* and *P. domestica* was also nearly same (Table 6). Further, for similar reasons as stated under TLC fingerprinting, the total fingerprint of the plants generated by HPLC was also evaluated and statistically analysed through similarity analysis for a suitable comparison. The chemometric analysis of the HPLC data using hierarchical clustering analysis placed *P. africana* and *P. domestica* in the same cluster indicating a close matching of their chemical profile.

The striking closeness of *P. domestica* with *P. africana* warranted investigation of the pharmacological activity to ascertain their efficacy in inflammation and oxidative stress. In this study we preferred to adopt a technique which is simple, economical, reproducible, does not involve many animals and can be easily adapted by especially resource constrained

countries and laboratories. The clinical studies have suggested a significant correlation between BPH, inflammation and oxidative stress for the development and progression of disease [47,48]. Both acute and chronic inflammation is believed to play a role in the proliferation of prostatic tissue through oxidative stress [67]. Phytosterols (β -sitosterol) of *Pygeum* extract reportedly inhibit the production of prostaglandins in the prostate, thereby suppressing the inflammatory symptoms associated with the BPH and chronic prostatitis [11,68]. Similarly, the pentacyclic triterpenes interfere by inhibiting the glucosyl-transferase activity, an enzyme involved in the inflammation process, while ferulic esters (*n*-docosanol) lower blood cholesterol levels from which testosterone is produced. Furthermore, phytosterols, triterpenes and ferulic esters are believed to work together in synergism against BPH [9,10,11]. The quantitative evaluation of the total phenolics, flavonoids and flavonols indicated that considerable quantities are present in all the species, with comparable amounts in *P. domestica* and *P. africana* (Tables 6 and 7). There is a well-established strong relationship between total phenolics and antioxidant activity [69]. It is noteworthy to associate the content of polyphenols and the total antioxidant potential of *Prunus* species because a linear correlation (Table 7) between total phenolic content and antioxidant activity was observed [70]. All the investigated species scavenged free radicals surpassing the standard drugs ascorbic acid and trolox. *P. domestica* showed lowest IC₅₀ value with an efficacy to suppress the oxidative stress more than *P. africana*. The presence of essential components and matching chemical profile complemented the results of *in vitro* anti-inflammatory studies as well, showing significant activity of the investigated *Prunus* species with *P. domestica* exhibiting higher membrane stabilization of RRBC than *P. africana* when compared to diclofenac.

In light of the results obtained out of pharmacognostic, chromatographic and biological studies of *Prunus* species, it is evidenced, that the investigated species of *Prunus* showed close relationship in bark characteristics and efficacy. Further, their usefulness in BPH has been recently established by us. It is meaningful to note that unlike *P. africana*, a good number of other *Prunus* species are known to be growing more widely across the globe, an aspect that will take care of their populations without exploitation. The scientifically planned approach of using *Prunus* species other

than *P. africana* will play a crucial role in its management. Such a strategy of validating the therapeutic efficacy of abundantly available analogous species would not only enhance the chances of success in terms of providing effective and safe drugs, but also is considered to go a long way in safeguarding the interests of pharmaceutical industry, biodiversity and society at large.

5. CONCLUSIONS

Regardless of all the advancement in modern healthcare systems, natural products are still an essential source of therapeutic measures, drug discovery and development. The different international agencies viz. World Health Organization, World Wildlife Fund, International Union for Conservation of Nature, International Plant Genetic Resources Institute and many national agencies play a crucial role in the conservation of natural sources. The present study focussed on providing a complete knowledge about the qualitative and quantitative comparison of bark of six species of *Prunus*. The investigated species are phytochemically related to the official drug *P. africana*. The higher phenolic/total sterol content, better antioxidant potential and membrane stabilization effect has validated the equivalence of potency in few of these species, especially *P. domestica* and *P. cerasoides* to that of *P. africana*, corroborating their possible use in BPH by suppressing inflammation and oxidative stress. It is expected that the study will be of immense value not only to African countries, but also to many countries world-over in exploring the usefulness of their indigenous species of *Prunus* as antiBPH agents and contribute towards the management plan of conservation of *P. africana*. Further, the study proposed a unique opportunity for employing the scientific research methods and policies to regulate preservation, cultivation, processing and marketing of the medicinal plants.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

ACKNOWLEDGEMENTS

The UGC-RFMS fellowship to authors AKJ and NS; UGC-MRP (43-504/2014(SR), instrument facility under DST-FIST/UGC-CAS programme; genuine sample of *Prunus africana* by Chemical Resources, Panchkula is duly acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kew Royal Botanical garden; 2016. Available:<http://www.kew.org/science-conservation/plants-fungi/environment/how-many-flowering-plants-are-there-world> Accessed 20 May 2016
2. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. *J. Nat. Prod.* 2016;79:629–661.
3. Stewart KM. The African cherry (*Prunus africana*): From hoe-handles to the international herb market. *Eco. Bot.* 2003; 57:559-569.
4. Pagano E, Laudato M, Griffo M, Capasso R. Phytotherapy of benign prostatic hyperplasia: A minireview. *Phytother. Res.* 2014;28:949-955.
5. Kalkman C. The old world species of *Prunus* subgenus *Laurocerasus* including those formerly referred to *Pygeum*. *Blumea.* 1965;13:1-115.
6. Watt JM, Beyer-Brandwijk MM. Medicinal and poisonous plants of Southern and Eastern Africa. E&S Livingstone Publishers, London; 1962.
7. Neuwinger HD. African traditional medicine: A dictionary of plant use and applications with supplements: Search system of diseases, Part 2. Medpharm Scientific Publishers, Stuttgart; 2000.
8. ESCOP Monographs. *Pygeum africanum* Cortex Monograph (2nd ed.). European Scientific Cooperative on Phytochemistry, Georg Thieme Verlag, Stuttgart Supplement; 2009.
9. Bombardelli E, Morazzoni P. *Prunus africana* (Hook. f.) Kalkm. *Fitoterapia.* 1997;68:205-218.
10. Awang DVC. Saw palmetto, African prune and stinging nettle for benign prostatic hyperplasia (BPH). *Can. J. Pharm.* 1997; 130:37-44.
11. Bassi P, Artibani W, De Luca V, Zattoni F, Lembo A. Standardized extract of *Pygeum africanum* in the treatment of benign prostatic hypertrophy. Controlled clinical study versus placebo. *Minerva Urol. Nefrol.* 1987;39:45-50.
12. Cunningham AB, Mbenkum FT. Sustainability of harvesting *Prunus africana* bark in Cameroon: A medicinal plant in international trade. UNESCO, Paris (People and Plants Working Paper 2); 1993.
13. Nkeng PF, Ingram V, Awono A. Assessment of *Prunus africana* bark exploitation methods and sustainable exploitation in the South west, North west and Adamaoua regions of Cameroon. Project GCP/ RAF/408/ EC. Mobilisation et Renforcement des Capacités des Petites et Moyennes Entreprises impliquées dans les Filières des Produits Forestiers Non Ligneux en Afrique Centrale. CIFOR. Yaoundé: FAO-CIFOR-SNV-World Agroforestry CenterCOMIFAC: 57; 2010.
14. Bodeker G, Klooster CV, Weisbord E. *Prunus africana* (Hook.f.) Kalkman: The over exploitation of a medicinal plant species and its legal context. *J. Altern. Complement. Med.* 2014;20:810-822.
15. Stewart KM. The African cherry (*Prunus africana*): Can lessons be learned from an over-exploited medicinal tree. *J. Ethnopharmacol.* 2003a;89:3-13.
16. Tewari K, Sharma A. World intellectual Property Organisation Patent No. 2013014681 A1. India; 2013.
17. Tewari K, Sharma A. U.S. Patent No. 20130236574 A1. India; 2013a.
18. Swaroop A, Bagchi M, Kumar P, Preuss HG, Bagchi D. Safety and efficacy of a novel *Prunus domestica* extract (Sitoprin, CR002) on testosterone-induced benign prostatic hyperplasia (BPH) in male Wistar rats. *Toxicol. Mech. Meth.* 2015;29:653-664.
19. Poonam V, Raunak Kumar G, Reddy LCS, Jain R, Sharma SK, Prasad AK, Parmar VS. Chemical constituents of the genus *Prunus* and their medicinal

- properties. *Curr. Med. Chem.* 2011;18:3758-3824.
20. Wiersema JH, Leon B. World economic plants - A standard reference. CRC Press. 1999;406-410.
 21. Hooker JD. Flora of British India, L. Reeve & Co. Ltd., The Oast House, Brook NR, Ashford, Kent. 1879;2:312-317.
 22. Pandey A, Nayar R, Venkateswaran K, Bhandari DC. Genetic resources of *Prunus* (Rosaceae) in India. *Genet. Resour. Crop Ev.* 2008;55:91-104.
 23. Kirtikar KR, Basu BD. Rosaceae, In: Blatter E., Caris, J.F., Mhaskar, K.S., (Eds.), *Indian Medicinal Plants*. Lalit Mohan Bash, Allahabad. 1984;951-965.
 24. Izzo R, Scartazza A, Masia A, Galleschi L, Quartacci MF, Navari-Izzo F. Lipid evolution during development and ripening of peach fruits. *Phytochemistry.* 1995;39: 1329-1334.
 25. Catalano S, Ferretti M. New constituents of *Prunus africana* bark extract. *J. Nat. Prod.* 1984;47:910.
 26. Hall JB, O'Brien EM, Sinclair FL. *Prunus africana*: A monograph. School of Agricultural and Forest Sciences Publication Number 18, University of Wales, Bangor. 2000;104.
 27. Cristoni A, Di Pierro F, Bombardelli E. Botanical derivatives for the prostate. *Fitoterapia.* 2000;71:S21-S28.
 28. Mwitari PG, Ayeka PA, Ondicho J, Matu EN, Bii CC. Antimicrobial activity and probable mechanisms of action of medicinal plants of Kenya: *Withania somnifera*, *Warbugia ugandensis*, *Prunus africana* and *Plectranthus barbatus*. *PLOS ONE.* 2013;8:e65619.
 29. Andro MC. *Pygeum africanum* extract for the treatment of patients with benign prostatic hyperplasia. *Curr. Ther. Res.* 1995;56:796-817.
 30. Rawat MSM, Pant G. A Persicogenin-3-glucoside from the stem bark of *Prunus amygdalus*. *Phytochemistry.* 1995; 38:1519-1520.
 31. Anonymous. The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products. New Delhi: National Institute of Science Communication and Information Resources. 2003;401-410.
 32. Catalogue of Life. Available:<http://www.catalogueoflife.org/annual> (Accessed 20 May 2016)
 33. Monumental Trees. Available:<http://www.monumentaltrees.com/en/deu-prunusarmeniaca> (Accessed 20 May 2016)
 34. AgroAtlas. Interactive Agricultural Ecological Atlas of Russia and Neighboring Countries. Available:http://www.agroatlas.ru/en/content/cultural/Prunus_armeniaca_K (Accessed 20 May 2016)
 35. Garg M, Garg SK, Gupta SR. Chemical examination of *Carum copticum* seeds and *Prunus cerasoids* stem bark. *Proc. Nat. Acad. Sci., India, Section A: Phy. Sci.* 1985;55:95-98.
 36. Shrestha PM, Dhillion SS. Medicinal plant diversity and use in the highlands of Dolakha district, Nepal. *J. Ethnopharmacol.* 2003;86:81-96.
 37. Pallavi GKL, Gupta V, Rishi R. Ethnopharmaco-botanical review of Padmaka – *Prunus puddum* Roxb. *Int. J. Ayur. Herb. Med.* 2011;1:87-99.
 38. Mahmood A, Ahmed R, Kosar S. Phytochemical screening and biological activities of the oil components of *Prunus domestica* Linn. *J. Saudi. Chem. Soc.* 2009;13:273-277.
 39. Tropicos. Available:<http://www.tropicos.org/Name/27800332?tab=distribution> (Accessed 20 May 2016)
 40. Canadensys. Available:<http://data.canadensys.net/vascan/taxon/8863> (Accessed 20 May 2016)
 41. Backheet EY, Farag SF. Flavonoids and cyanogenic glycosides from the leaves and stem bark of *Prunus persica* (L.) Batsch. (Meet ghamr) peach local cultivar in Assuit region. *Bull. Pharm. Sci.* 2003;26: 55-56.
 42. Aziz S, Rahman H. Biological activities of *Prunus persica* L. batch. *J. Med. Plants Res.* 2013;7:947-951.
 43. World Health Organisation. WHO monographs on selected medicinal plants. 2001;3:64-76.
 44. Rokayaa MB, Münzbergovaa Z, Timsina B. Ethnobotanical study of medicinal plants from the Humla district of western Nepal. *J Ethnopharmacology.* *J. Ethnopharmacol.* 2010;130:485-504.

45. Zaidi SF, Muhammad JS, Shahryar S, Usmanghani K, Gilani A, Jafri W, Sugiyama T. Anti-inflammatory and cytoprotective effects of selected Pakistani medicinal plants in *Helicobacter pylori*-infected gastric epithelial cells. J. Ethnopharmacol. 2012;141:403-410.
46. Sreekeesoon DM, Mahomoodally MF. Ethnopharmacological analysis of medicinal plants and animals used in the treatment and management of pain in Mauritius. J. Ethnopharmacol. 2014;157: 181–200.
47. Chughtai B, Lee R, Te A, Kaplan S. Role of inflammation in benign prostatic hyperplasia. Rev. Urol. 2011;13:147-150.
48. Kramer G, Marberger M. Could inflammation be a key component in the progression of benign prostatic hyperplasia? Curr. Opin. Urol. 2006;16:25-29.
49. Robert G, Descazeaud A, Nicolaiew N, Terry S, Sirab N, Vacherot F, Maille P, Allory Y, de la Taille A. Inflammation in benign prostatic hyperplasia: A 282 patients' immunohistochemical analysis. Prostate. 2009;69:1774-1780.
50. Jena AK, Vasisht K, Sharma N, Kaur R, Dhingra MS, Karan M. Amelioration of testosterone induced benign prostatic hyperplasia by *Prunus* species. J. Ethnopharmacol. 2016;190:33-45.
51. World Health Organisation. Quality control methods for medicinal plant materials. WHO Press, Geneva; 1998.
52. Tawaha K, Alali FQ, Gharaibeh M, Mohammad M, El-Elimat T. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem. 2007;104:1372-1378.
53. Kumaran A, Karunakaran J. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT Food Sci. Technol. 2007;40:344-352.
54. Abdel-Hameed ES. Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples. Food Chem. 2009;114:1271-1277.
55. Larissa BDCA, Sarah LS, Marcos AMG, Magda RAF, Evani LA, Karina PR, Luiz ALS. Total phytosterol content in drug materials and extracts from roots of *Acanthospermum hispidum* by UV-VIS spectrophotometry. Rev. Bras. Farmacogn. 2013;23:736-742.
56. Kenny AP. The determination of cholesterol by the Liebermann-Burchard reaction. Biochem. J. 1952;52:611-619.
57. Awe EO, Makinde JM, Adeloye OA, Banjoko SO. Membrane stabilizing activity of *Russelia equisetiformis*, Schlecht & Chan. J. Nat. Prod. 2009;2:3-9.
58. Perez RM, Perez S, Zavala, MA, Salazar M. Anti-inflammatory activity of the bark of *Hippocratea excels.* J. Ethnopharmacol. 1995;47:85-90.
59. Vasisht K, Chadha K, Karan M, Bhalla Y, Jena AK, Chadha R. Enhancing biopharmaceutical parameters of bioflavonoid quercetin by cocrystallization. Cryst. Eng. Comm. 2016;18:1403-1415.
60. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 1995;28:25-30.
61. Re R, Pellegrini N, Proreggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 1999;26:1231-1237.
62. Zhang Z, Chen G, Zhou W, Song A, Xu T, Luo Q, Wang W, Gu XS, Duan S. Regulated ATP release from astrocytes through lysosome exocytosis. Nat. Cell Biol. 2007;9:945-953.
63. Saleem TKM, Azeem AK, Dilip C, Sankar C, Prasanth NV, Duraisami R. Anti-inflammatory activity of the leaf extracts of *Gendarussa vulgaris* Nees. Asian Pac. J. Trop. Biomed. 2011;1:147-149.
64. Vinceti B, Loo J, Gaisberger H, van Zonneveld MJ, Schueler S, Konrad H, Kadu CAC, Geburek T. Conservation priorities for *Prunus africana* defined with the aid of spatial analysis of genetic data and climatic variables. PLOS ONE. 2013;8:e59987-e60002.
65. Jimu L. Threats and conservation strategies for the African cherry (*Prunus africana*) in its natural range-a review. J. Ecol. Nat. Environ. 2011;3:118-130.
66. Jena AK, Karan M, Vasisht K. Plant parts substitution based approach as a viable conservation strategy for medicinal plants: A case study of *Premna latifolia* Roxb. J. Ayurveda. Integr. Med. 2017;8:68-72.
67. Naber KG, Weidner W. Chronic prostatitis - an infectious disease? J. Antimicrob. Chemother. 2000;46:157-161.
68. Breza J, Dzurny O, Borowka A, Hanus T, Petrik R, Blane G, Chadha-Boreham H.

- Efficacy and acceptability of tadenan (*Pygeum africanum* extract) in the treatment of benign prostatic hyperplasia (BPH): A multicentre trial in central Europe. *Curr. Med. Res. Opin.* 1998;14: 127-139.
69. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* 1998;46: 4113-4117.
70. Gheldof N, Engeseth NJ. Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of *in vitro* lipoprotein oxidation in human serum samples. *J. Agric. Food Chem.* 2002;50:3050-3055.

© 2017 Karan et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://sciencedomain.org/review-history/20771>