ISSN 1996-3351

Asian Journal of Biological Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2016.19.26

Research Article
Pharmacognostical, Antibacterial and Antioxidant Studies of Aerial Parts of *Pulicaria somalensis* (Family: Asteraceae)

[1]Ahmed I. Foudah, [1]Aftab Alam, [2,3]Gamal A. Soliman, [1]Mohammed Ayman Salkini, [4]Elmutasim O. Ibnouf Ahmed and [1]Hasan S. Yusufoglu

[1]Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia
[2]Department of Pharmacology, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia
[3]Department of Pharmacology, College of Veterinary Medicine, Cairo University, Egypt
[4]Department of Pharmaceutics, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia

Abstract
The study was designed to investigate the pharmacognostic parameters of the aerial parts as well as antimicrobial and antioxidant activities of methanol extract of *Pulicaria somalensis* O. Hoffm (MEPS). These studies were carried with a view to justify the future use of this plant. This study deals with the morphological, microscopical studies of leaf, aerial part of *P. somalensis*, along with the physicochemical and preliminary phytochemical analyses that were also studied. The antibacterial activities were tested against both Gram-positive and Gram-negative bacteria while antifungal activities were tested against *Aspergillus niger* and *Candida albicans* using the agar disc diffusion method. The zone of inhibition was compared with standard (Ampicillin). The study of *in vitro* antioxidant was performed by using DPPH and FRAP assays. The results of the present morphological study correlated with earlier reports. The microscopical and physicochemical finding explores the useful identification character for authentication of this plant. The preliminary phytochemical study showed the presence of phenol, tannins and flavonoids types of active drugs. The marked antibacterial, antifungal and antioxidant were observed, which may be due to the presence of active constituents present in MEPS. The outcome of the present findings suggested that the plant may be a good source of antibacterial and antioxidant. The present finding concluded the *in vitro* activities, so it needs to be explored more *in vivo* evaluation before adding it into the world of medicinal plants.

Key words: Pharmacognostic, *Pulicaria somalensis*, phytochemical screening, antimicrobial, antioxidants

Received: October 11, 2015 **Accepted:** November 26, 2015 **Published:** March 15, 2016

Citation: Ahmed I. Foudah, Aftab Alam, Gamal A. Soliman, Mohammed Ayman Salkini, Elmutasim O. Ibnouf Ahmed and Hasan S. Yusufoglu, 2016. Pharmacognostical, antibacterial and antioxidant studies of aerial parts of *Pulicaria somalensis* (Family: Asteraceae). Asian J. Biol. Sci., 9: 19-26.

Corresponding Author: Hasan S. Yusufoglu, Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173 Al-Kharj, 11942, Kingdom of Saudi Arabia Tel: 00966-11-588-6012 Fax: 00966-11-588-6001

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plant *P. somalensis* (family-Asteraceae), distributed all over the world, especially in North America, Europe, Southern Africa, South Western China, Central Asia and Mediterranean region except, of Antarctica continent (Funk *et al*., 2005). The family Asteraceae, which contains about 1,620 genera and 23,600 species, is one of the largest angiosperm plant families among the dicotyledonous (Stevens, 2013). Few plants of this family are shrubs, creepers and climber but the majority of plants of this family is herbaceous in nature. There are several key elements present in this family, such as fused anthers, fruits with single ovules and capitulum inflorescence, which are used for the detection of their species (Garcia *et al*., 2010). Many members of this rich family have long been used in folk medicines (Chhetri *et al*., 2015). Antibiotics were widespread in natural crude drugs and exercised since their innovation in the 20th century. However, the emergence of multi-drug resistant pathogens, it becomes a global challenge to the medical world to investigate an active antibiotic (Balkhair *et al*., 2014). It is now widely recognized that there is a need to develop novel antibiotic agents to minimize the risk of antimicrobial resistance. Most of the species of this genus have been well explored for their medicinal values. The standardization of *P. somalensis* can be achieved by stepwise pharmacognostic studies (Chanda, 2014). Accurate identification and quality assertion of the preliminary materials are essential requirements to ensure the reproducible quality of herbal medicine, which will contribute to its safety and efficacy. The simple pharmacognostic method used in standardization of plant material includes its morphological and anatomical characters (Fazal *et al*., 2013). Oxidative damage has a key role in the progress of several pathogenic diseases. Antibacterial activities prevent several pathogenic diseases because bacteria (aerobic) have redox enzymes that mediate oxidative phosphorylation with an oxygen molecule, produce ROS (reactive oxygen species) (Kashmiri and Mankar, 2014). *Pulicarias somalensis* are wildly grown in Al-Kharj region but till this time as per our knowledge, it was not explored for its medicinal importance. With this in mind, a study was designed to explore the morphological, microscopical, physicochemical and phytochemical aspects of aerial parts of *P. somalensis* with the antimicrobial and antioxidant evaluations of its methanolic extract.

MATERIALS AND METHODS

Materials: All the chemical and reagents procured were of analytical grade, phloroglucinol was procured from lobachemie. Glycerol (90% purified), glacial acetic acid (AR Grade), ethanol (AR Grade), potassium iodide (AR Grade), lead acetate (AR Grade), DPPH, trichloroacetic, potassium ferricyanide were from Sigma Aldrich.

Plant material: The aerial parts of *Pulicaria somalensis* was collected in early march 2014 from the new industrial area 17 km south west Alkharj city. The collected plant was authenticated by taxonomist Dr. M. Atiqur Rahman, from College of Pharmacy, Medicinal, Aromatic and Poisonous Plants Research Center, King Saud University, Riyadh. A voucher specimen (PSAU-CPH-3-2014) is maintained in the herbarium of College of Pharmacy, Prince Sattam Bin Abulaziz University. The plant material was air dried and reduced to fine powder.

Pharmacognostical standardization

Macro and microscopic examinations of powder and leaf section: For the macroscopic aerial parts were used and for the microscopy, leaf was dipped in chloral hydrate solution for several hours until it lost its color and pigments, after which transverse sections were cut at the lower third of the leaves (Kumar *et al*., 2012). In short, the leaf section was mounted on slides and placed under the microscope. The microscopy of powder was done using a compound microscope (Inco-Ambala) and photographs were taken using photographic microscope (Motic-Image-2003) (Yusufoglu, 2015). In short, a glass slide was taken and a pinch of powdered material was placed on the slide after adding chloral hydrate and heating a specimen was prepared. This specimen was then placed and observed under the magnifying lenses of 10, 40 and 100x magnification of the microscope.

Ash, moisture and extractive content of powder drugs: The physicochemical parameters such as Ash (total ash, water soluble ash, acid insoluble ash), moisture and the extractive (hexane, chloroform, methanol and water) content were carried out following the reported methods (Kokate *et al*., 1994; Ajazuddin and Saraf, 2010).

Preliminary phytochemical screening: The preliminary phytochemical tests were carried out of powdered specimens using standard procedures (Doss, 2009; Evans, 2006).

Antimicrobial studies

Preparation of sample: The Methanol Extract of *P. somalensis* (MEPS) was prepared by soaking 200 g dried powder in 2 L of

methanol for 24 h. The extracts were filtered and evaporate under reduced pressure using a rotary evaporator.

Microorganisms

Three gram-positive bacteria: *Staphylococcus aureus* (ATCC 29213), *Staphylococcus aureus* (clinical isolate) and *Bacillus subtilis* (ATCC 10400) and three Gram negative bacteria namely *Escherichia coli* (ATCC 10536), *Klebsiella pneumonia* (ATCC 13882) and *Proteus vulgaris* (clinical isolate) were used for antibacterial study. *Aspergillus niger* (ATCC 16404) and *Candida albicans* (NCYC 1363) were used for anti-fungal study. All the microbial strains were obtained from College of Pharmacy, Microbiology Research Lab, Prince Sattam Bin Abdulaziz University, Al-Kharj. The selection of the microbial strains was based on their pharmacological and clinical relevance (McCracken and Cowsan, 1983). The bacterial strains were grown in Mueller-Hinton agar (Oxoid, Basingstoke, England) plates at 37°C and the stock cultures were maintained at 4°C. The fungal strains were subcultured in PDA (Potato dextrose agar) media at 25°C and the stock cultures were maintained at 4°C.

Antibacterial and antifungal activities of MEPS against six pathogenic bacteria (three Gram-positive and three Gram-negative) and two pathogenic fungi were investigated by the agar disk diffusion method (Rios *et al*., 1988). The MEPS was dissolved in dimethyl sulfoxide, sterilized by filtration using a sintered glass filter and stored at 4°C. The test (MEPS, 100 μg mL^{-1}) and standard drugs (Ampicillin, 10 μg) were prepared in double-distilled water. Mueller-Hinton sterile agar plates were seeded with bacterial strains (10^8 cfu) and allowed to stay at 37°C for 3 h. On solidification, 5 mm holes were made with sterile borer and 0.25 mL of the test strains were inoculated in the media separately. The zones of growth inhibition around the disks were measured after 24 h of incubation at 37°C for bacteria and 48 h for fungi at 28°C (Jayashree and Londonkar, 2014). The sensitivities of the microorganism species were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks and values <8 mm were considered as not active against microorganisms.

Antioxidant evaluation

DPPH radical scavenging assay: The DPPH radical scavenging of MEPS was done using the method of Ebrahimzadeh *et al*. (2008). The DPPH radicals showed a strong absorption at 517 nm, color changed from purple to yellow, the absorbance decreased with reduction by an antioxidant compound (s). A portion (1 mL) of each of the different concentrations (10-1000 μg mL^{-1}) of the extracts or ascorbic acid (standard) was added to 1 mL of 1 mmol L^{-1} DPPH in methanol. The reaction mixtures were vortex and incubated in the dark for 30 min, after which the absorbance was measured against control (DPPH having 1 mL of methanol in place of the extract). The experiment was carried out in triplicate. Percentage radical scavenging activity of DPPH was calculated using the following formula (Yusufoglu *et al*., 2015):

$$\text{DPPH scavenging effect (\%)} = (CA - SA)/CA) \times 100$$

where, CA = Control absorption and SA Sample absorption.

Ferric-reducing power assay: This was determined according to the method by Yusufoglu *et al*. (2015). One milliliter of each of the MEPS or standard (100 μg mL^{-1}) with different concentrations (250-2000 μg mL^{-1}) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and cooled. Trichloroacetic acid (10%, 2.5 mL) was added to the mixture and the content was centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with $FeCl_3$ (0.1%, 0.5 mL) and distilled water (2.5 mL). The absorbance was measured at 700 nm in a UV spectrophotometer. The increasing, reducing power was indicated by increasing absorbance of the reaction mixture.

Statistical analysis: Results are expressed as Mean ± Standard Error (SE) of mean. Statistical analysis was performed, using one-way analysis of variance (ANOVA). When the F-value was found statistically significant ($p<0.05$), further comparisons among groups were made using Dunnett's multiple comparisons test. All statistical analyses were performed using SPSS version 17.0.

RESULTS

Pharmacognostical standardization: The macroscopic studies of the *P. somalensis* (Fig. 1) were performed. The studies showed that plant contains, lanceolate leaves, bushy stem and tap root. The flowers were containing yellow corolla, capitula in small corymbs. The powder of dried plants was yellowish in color with stringent taste and aromatic flavor (Table 1). The microscopic characters of powder were observed and revealed that epidermis with stomata (Anomocytics), trichomes, pollen grains (spiny) (Fig. 2). Leaf sections of this plant showed that the upper epidermis has a thick cuticle with a single layer. Lower epidermis was also

Fig. 1: Arial part of *Pulicaria somalensis*

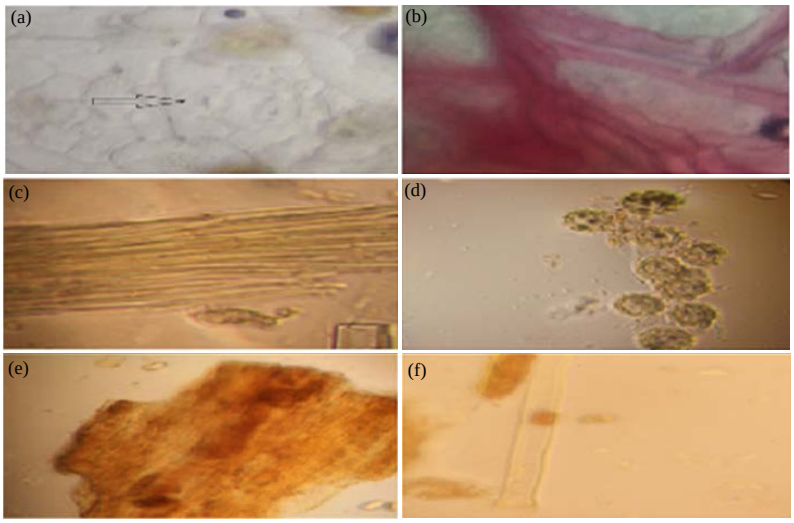

Fig. 2(a-f): Powder microscopy study (×40), (a) Epidermal cell with stomata, (b) Spiral vessels, (c) Fibers, (d) Pollen grains, (e) Tannin containing cells and (f) Long glandular trichome

Table 1: Macroscopic study of *Pulicaria somalensis* herb

Structure	Colour	Types
Leaf	Yellow-green	Lanceolate
Stem	Yellow-green	Branched
Root	Yellow-white	Taproot
Flower	White and yellowish	Capitula in small corymbs, corolla yellow
Powder	Yellowish colour	Coarse
Taste	-	Stringent
Flavor	-	Aromatic

-: Absent

single-layered with stomata and unicellular simple trichomes (Fig. 3). The study of physicochemical parameters such as total ash (1.05%), the moisture content (1.59%) and water soluble extractive values (14.25%) revealed the presence of sugar, acids and inorganic compounds (Table 2). The plant powder was subjected to the preliminary phytochemical studies and results were tabulated and revealed that the major categories of phytochemicals were present (Table 3).

Antimicrobial studies: The results of the zone of inhibition, which is the parameter observed for antibacterial activity of standard drug (Ampicillin) and MEPS. The MEPS had good inhibitory action on *K. pneumoniae* (23 mm), *B. subtilis*

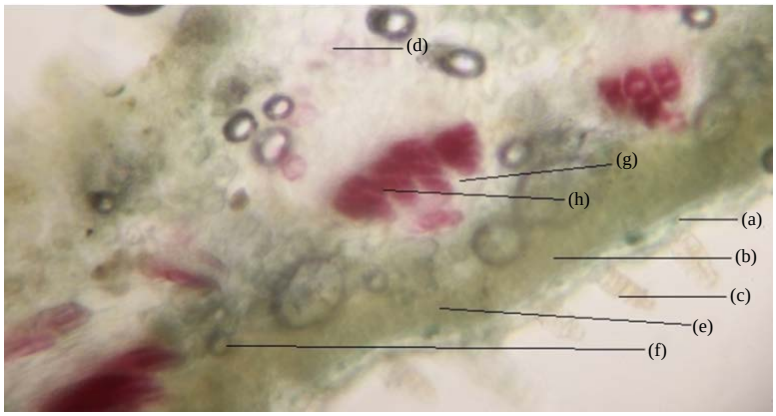

Fig. 3: *Pulicarias somalensis* leaf (T. S.) at 20X, a: Cuticle, b: Epidermis, c: Trichome, d: Stomata, e: Palisade mesophyll, f: Spongy mesophyll, g: Phloem and h: Xylem

Table 2: Physicochemical evaluations of aerial parts powder *Pulicaria somalensis*

Parameter and result	Percentage values	Colour
Ash value		
Total ash (%w/w)	1.05	-
Acid insoluble ash (%w/w)	0.88	
Water soluble ash (%w/w)	0.25	
Percentage moisture content		
Moisture content	1.59	-
Percentage extractive value		
Hexane	2.55	Light yellow
Chloroform	5.80	Yellow
Methanol	7.10	Green
Distilled water	14.25	Yellow brown

-: Absent

Table 3: Preliminary phytochemical study of aerial parts powder of *P. somalensis*

Phytoconstituents and test	Result
Alkaloids	
Mayer's test	-
Wagner's test	++
Dragendroff's test	+
Carbohydrates	
Molisch's test	++
Benedict's test	+
Fehling's test	++
Glycosides	
Modified borntrager's test	+
Keller-Killiani test	-
Saponins	
Foam test	++
Test for steroids and triterpenoids	
Salkowski's test	++
Liebermann burchard's test	++
Fats and oils	
Stain test	+
Phenols and tannins	
Ferric chloride test	++
Flavonoids	
Lead acetate test	+
Proteins and aminoacids	
Ninhydrin test	+
Biuret test	+

+: Present, ++: Strongly present, -: Absent

(16 mm) and *E. coli* (25 mm) but less when compared to that of the standard (Table 4). The antifungal activities were also observed. The standard solution has good inhibitory action on *C. albicans* (28 mm) and MEPS (18 mm) was comparatively less active. It was also found that MEPS solution has comparatively less inhibitory effect on *A. niger* (15 mm) when compared to standard (23 mm). No action on the three strains *S. aureus* (ATCC 29213), *S. aureus* (clinical isolate), *P. vulgaris* (clinical isolate) was seen.

Antioxidant evaluation: In DPPH assay, MEPS demonstrated a significant antioxidant potential in a concentration dependent manner (Fig. 4). The higher percentage inhibition of ascorbic acid account the better antioxidant potential, compared to MEPS. MEPS at concentrations of 10, 50, 100, 500 and 1000 μg mL^{-1} showed scavenging activities of 19.57, 41.40, 76.78, 81.23 and 79.84%, respectively, while ascorbic acid scavenging activities showed 48.65, 71.90, 84.62, 92.56 and 96.51%, respectively.

In present ferric-reducing power assay ($Fe^{3+}$to Fe^{2+}), MEPS increased with the concentration of the extract. The reducing power of ascorbic acid (Standard) was found to be higher than MEPS. MEPS at concentrations of 250, 500, 1000 and 2000 μg mL^{-1} showed average reducing activities of 0.555, 0.897, 1.744 and 2.618, respectively, while ascorbic acid average reducing activities showed 1.598, 1.878, 2.768 and 2.967, respectively (Fig. 5).

DISCUSSION

In present macroscopic finding, it was found that the plant is yellow to green in color with lanceolate leaves, bushy

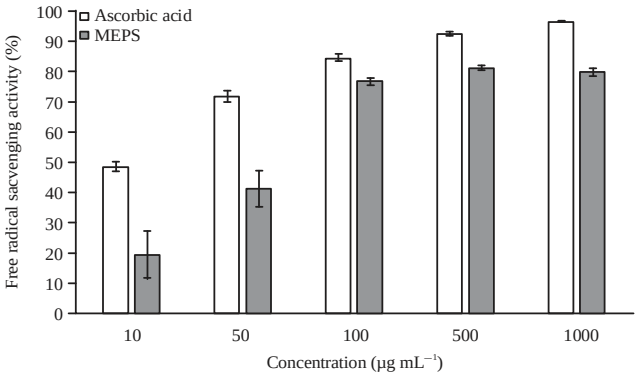

Fig. 4: DPPH activity of MEPS and ascorbic acid

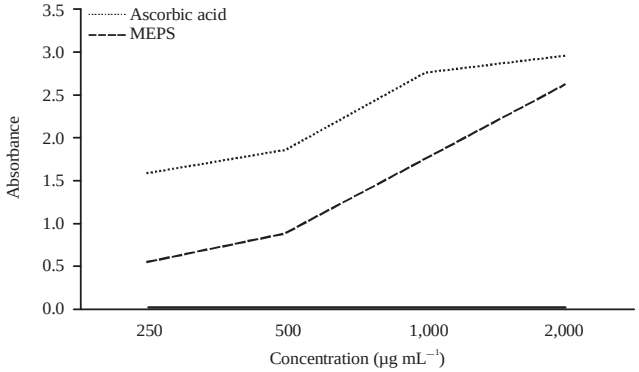

Fig. 5: Reducing (Ferric) assay of MEPS and ascorbic acid

Table 4: Zone of inhibition (mm) analysis of MEPS

	S. aureus							
Sample	ATCC	CI	*B. subtilis* ATCC	*E. coli* ATCC	*K. pneumonia* ATCC	*P. vulgaris* (CI)	*A. niger* (ATCC)	*C. albicans* (NCYC)
MEPS	NA	NA	16	17	23	NA	15	18
Amp. (10 µg)	NA	NA	25	25	33	NA	23	28

*CI: Clinically isolated Munich, Amp: Ampicillin, NA: Not active, mm: Millimeter

stem and tap root. The flower of this plant is white and yellowish in color with yellow corolla, capitula in small corymbs. Some of the morphological characters of this plant has been explained as a shrub or woody herb containing lanceolate leaf, acute, glabrous or pilose (http://plants.jstor.org). Leaf and powder microscopy were studied in details to highlight the important anatomical characters of this plant. In the present finding, the microscopic characters of powder revealed that it contains anomocytics types of stomata, long glandular trichome and spiny pollen grains. The leaf sections showed thick cuticle with single-layered upper epidermis and the lower epidermis with stomata and unicellular simple trichomes. The microscopical characters of present study may use to identify the adulteration of the plant powdered. At present, instead of having sophisticated advanced research tools for estimation of the plant drugs, the old microscopic procedure is still one of the simplest and cheapest methods for identifying the adulterants (Singh *et al*., 2010). The physicochemical parameters such as ash values of a drug give an idea of the inorganic and other impurities present along with the powdered drug, moisture and extractive values, which are primarily useful for the detection of adulterated drugs (Singhal *et al*., 2010). The phytochemical study of this plant revealed the presence of flavonoids, phenolic and tannins. These compounds are known to reveal a wide range of biological activities like hepatoprotective, cardioprotective, neuroprotective, cytotoxic, anticancer, antimicrobial, antiviral and antioxidant properties (Havsteen, 2002). The antimicrobial activity of medicinal plant extracts has been attributed to the presence of phytochemical compounds like Saponins, tannins and flavonoids (Murugan *et al*., 2013). The presence of these

compounds usually justifies the use of the plants for treatment of infections caused by pathogens. The MEPS have shown antibacterial and antifungal activities, the result displayed appreciable antimicrobial activities against *K. pneumoniae*, *B. subtilis* and *E. coli* but comparatively *B. subtilis* and *E. coli* showed the less active than *K. pneumoniae*. These microorganisms are well documented for different types of infectious diseases (Al-Bayati and Al-Mola, 2008). The MEPS showed better inhibitory action on *C. albicans* than *A. niger*. The *Candida* species are the most common cause of fungal infections of a range of life threatening persistent to non-life-threatening muco-cutaneous diseases and among *Candida* species, *C. albicans* is the most common infectious agent (Achkar and Fries, 2010).

The DPPH antioxidant activity was employed to determine the scavenging potentials of MEPS. DPPH (α, α-diphenyl-β-picrylhydrazyl) is converted into α, α-diphenyl-β-picrylhydrazine with color change during the free radical reaction. The rate of color change gradually decreased, which indicates the scavenging potentials of the sample antioxidant. The phytochemical screening of MEPS was confirmed due to the presence of Saponins, tannins, flavonoid and phenolics and was able to discolor DPPH solution with their hydrogen donating ability (Alabri *et al*., 2014; Waheed *et al*., 2014).

The FRAP antioxidant activity was employed to determine the reducing potential of MEPS. FRAP (Yellow, ferric salt) is converted into a ferrous complex (blue colored) during the reducing activity. In the reaction mixture, the absorption change was linked directly with the total reducing power of antioxidant compounds, which reduced the Ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion (Sudan *et al*., 2014). Flavonoids and phenolic contents exhibited an important antioxidant, the highest FRAP activity in the MEPS, signifying the presence of richer flavonoids and phenolic phytochemicals antioxidants and reductants (Alafiatayo *et al*., 2014).

CONCLUSION

Pharmacognostical and physiochemical standards of *P. somalensis* were explored for the first time and the present finding can be useful to validate and authenticate this plant. Results of the present antimicrobial studies conclude that MEPS work against both bacteria and fungi. The antioxidant capacity of MEPS showed the strongest free radical scavenging activities with high antioxidant reductants. The presence of flavonoids, tannins and phenolic compounds is responsible for both antimicrobial and antioxidant activities. This study has provided evidence that *P. somalensis* is a potential source of antimicrobials and antioxidants and could be a future drug.

ACKNOWLEDGMENTS

This project was supported by Support Research Projects with New Faculty Grant Program, Deanship of Scientific Research, Prince Sattam Bin Abdulaziz University, Al-Kharj, KSA, Grant No. 2015/03/4023. The authors would like to express their gratitude to the Deanship of Scientific Research of Prince Sattam Bin Abdulaziz University, Al-Kharj, for their assistance.

REFERENCES

Achkar, J.M. and B.C. Fries, 2010. *Candida* infections of the genitourinary tract. Clin. Microbiol. Rev., 23: 253-273.

Ajazuddin and S. Saraf, 2010. Evaluation of physicochemical and phytochemical properties of Safoof-E-Sana, a Unani polyherbal formulation. Pharmacogn. Res., 2: 318-322.

Al-Bayati, F.A. and H.F. Al-Mola, 2008. Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. J. Zhejiang Univ. Sci. B, 9: 154-159.

Alabri, T.H.A., A.H.S. Al Musalami, M.A. Hossain, A.M. Weli and Q. Al-Riyami, 2014. Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. J. King Saud Univ. Sci., 26: 237-243.

Alafiatayo, A.A., A. Syahida and M. Mahmood, 2014. Total anti-oxidant capacity, flavonoid, phenolic acid and polyphenol content in ten selected species of Zingiberaceae rhizomes. Afr. J. Tradit. Complement. Altern. Med., 11: 7-13.

Balkhair, A., Y.M. Al-Farsi, Z. Al-Muharrmi, R. Al-Rashdi and M. Al-Jabri *et al*., 2014. Epidemiology of multi-drug resistant organisms in a teaching hospital in Oman: A one-year hospital-based study. Sci. World J. 10.1155/2014/157102

Chanda, S., 2014. Importance of pharmacognostic study of medicinal plants: An overview. J. Pharmacogn. Phytochem., 2: 69-73.

Chhetri, B.K., N.A.A. Ali and W.N. Setzer, 2015. A survey of chemical compositions and biological activities of Yemeni aromatic medicinal plants. Medicines, 2: 67-92.

Doss, A., 2009. Preliminary phytochemical screening of some Indian Medicinal Plants. Anc. Sci. Life, 29: 12-16.

Ebrahimzadeh, M.A., F. Pourmorad and S. Hafezi, 2008. Antioxidant activities of Iranian corn silk. Turk. J. Biol., 32: 43-49.

Evans, W.C., 2006. Trease and Evans Pharmacognosy. 15th Edn., Rajkamal Electric Press, New Delhi, India, pp: 513-547.

Fazal, H., N. Ahmad and B.H. Abbasi, 2013. Identification, characterization and palynology of high-valued medicinal plants. Sci. World J. 10.1155/2013/283484

Funk, V.A., R.J. Bayer, S. Keeley, R. Chan and L. Watson *et al*., 2005. Everywhere but Antarctica: Using a supertree to understand the diversity and distribution of the Compositae. Biologiske Skrifter, 55: 343-374.

Garcia, S., J.L. Panero, J. Siroky and A. Kovarik, 2010. Repeated reunions and splits feature the highly dynamic evolution of 5S and 35S ribosomal RNA genes (rDNA) in the Asteraceae family. BMC Plant Biol., Vol. 10. 10.1186/1471-2229-10-176

Havsteen, B.H., 2002. The biochemistry and medical significance of the flavonoids. Pharmacol. Therapeut., 96: 67-202.

Jayashree, V.H. and R. Londonkar, 2014. Comparative phytochemical studies and antimicrobial potential of fruit extracts of *Feronia limonia* Linn. Int. J. Pharm. Pharmaceut. Sci., 6: 731-734.

Kashmiri, Z.N. and S.A. Mankar, 2014. Free radicals and oxidative stress in bacteria. Int. J. Curr. Microbiol. Applied Sci., 3: 34-40.

Kokate, C.K., A. Purohit and S. Gokhale, 1994. Practical Pharmacognosy. 10th Edn., Vallabh Prakashan, New Delhi, India, pp: 112-114.

Kumar, D., K. Kumar, S. Kumar, T. Kumar, A. Kumar and O. Prakash, 2012. Pharmacognostic evaluation of leaf and root bark of *Holoptelea integrifolia* Roxb. Asian Pac. J. Trop. Biomed., 2: 169-175.

McCracken, W.A. and R.A. Cowsan, 1983. Clinical and Oral Microbiology. Hemispher Publishing Corporation, New York, USA, Pages: 512.

Murugan, T., J.A. Wins and M. Murugan, 2013. Antimicrobial activity and phytochemical constituents of leaf extracts of *Cassia auriculata*. Indian J. Pharmaceut. Sci., 75: 122-125.

Rios, J.L., M.C. Recio and A. Villar, 1988. Screening methods for natural products with antimicrobial activity: A review of the literature. J. Ethnopharmacol., 23: 127-149.

Singh, S., L. Machawal and M.G. Chauhan, 2010. Pharmacognostic study of male leaves of *Trichosanthes dioica* Roxb. with special emphasis on microscopic technique. J. Pharma. Phytother., 2: 71-75.

Singhal, A.K., V.S. Bhati and V.K. Singhal, 2010. Pharmacognostic study of aerial parts of plant *Geniosporum prostratum* (L.) Benth. J. Scient. Speculations Res., 1: 19-24.

Stevens, P.F., 2013. Angiosperm phylogeny website, version 13. http://www.mobot.org/ MOBOT/ research/APweb/

Sudan, R., M. Bhagat, S. Gupta, J. Sing and A. Koul, 2014. Iron (FeII) chelation, ferric reducing antioxidant power and immune modulating potential of *Arisaema jacquemontii* (Himalayan Cobra Lily). BioMed. Res. Int. 10.1155/2014/179865

Waheed, I., M. Ahmad, N.H. Syed and R. Ashraf, 2014. Investigation of phytochemical and antioxidant properties of methanol extract and fractions of *Ballota limbata* (Lamiaceae). Indian J. Pharm. Sci., 76: 251-256.

Yusufoglu, H.S., 2015. Pharmacognostic and wound healing studies of the leaves of *Bassia eriophora* (Family: Chenopodiaceae) on albino rats. Annu. Res. Rev. Boil., 5: 400-408.

Yusufoglu, H.S., G.A. Soliman, R.F. Abdel-Rahman, M.S. Abdel-Kader and M.A. Genaie *et al*., 2015. Antioxidant and antihyperglycemic effects of *Ferula drudeana* and *Ferula huber-morathii* in experimental diabetic rats. Int. J. Pharmacol., 11: 738-748.