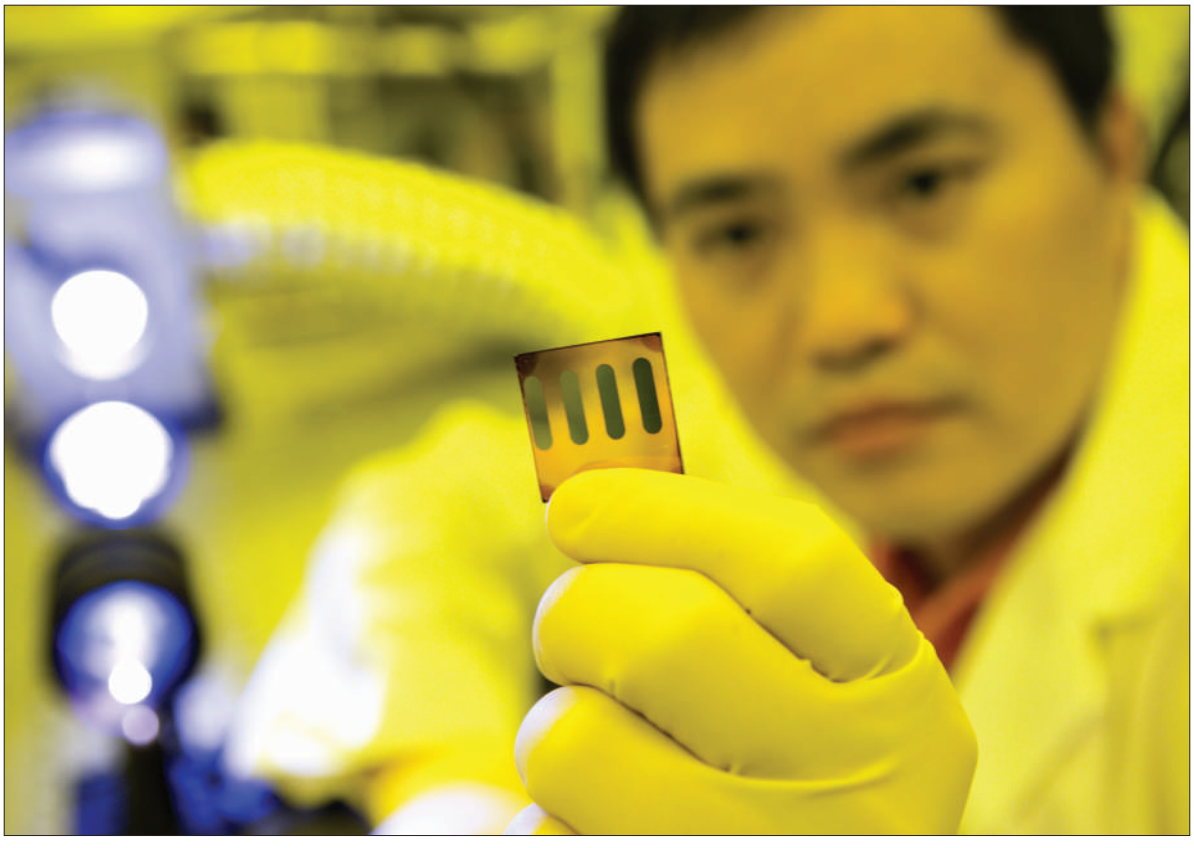

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Pharmacognostical Evaluation of Ethanolic, Petroleum Ether and Distilled Water Extract of *Woodfordia fruticosa*, *Cissampelos pareira* and *Stephania glabra*

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ABSTRACT

The idea for the present study is taken up from the folklore uses of *Woodfordia fruticosa* (WFE), *Cissampelos pareira* (CPE) and *Stephania glabra* (SGE). These plants are used to treat different ailments in tribal areas of Nepal, Himalayan region of India and China. Knowing their historical medicinal value, present study is focused on pharmacognostical study of different extracts from leaves of WFE, CPE and rhizome of SGE. Powder of dried leaves of WFE and CPE and rhizome of SGE were extracted by soxhlet apparatus using petroleum ether and ethanol as solvent. These plants were also extracted with distilled water by maceration. Thin Layer Chromatography (TLC) was performed for distilled water extracts, Hydrodistillation of 30 g dried leaves powder of CPE were performed by using clevenger apparatus. The phytochemical screening showed the presence of flavonoids, carbohydrate in all the extracts. The amount of volatile oil was reported to be 0.33%. R_f values in TLC plates from solvent system Toluene: Ethyl acetate was reported as 0.18, 0.077, 0.155 for SGE, 0.166, 0.24 for WFE and 0.5 and 0.488 for CPE. R_f values from solvent system Hexane: Chloroform: Methanol were reported as 0.126, 0.294, 0.642, 0.631 and 0.894 from CPE. In this solvent system R_f values for WFE were reported as 0.085, 0.0571, 0.042 and for SGE as 0.075, 0.1, 0.125, 0.15, 0.187 and 0.212. From the above study it was concluded that these studies could be useful for identification and preparation of a monograph of these plants.

Key words: Physicochemical, phytochemical, hydrodistillation

INTRODUCTION

Plant derived drug has gained a good reputation both in traditional and modern medicine (Das *et al*., 2007; Akerele, 1993). Most of the medicinal plants from tribal origin with ethnopharmacological and ethnobotanical interest lack preclinical scientific validations (Baker and Alvi, 2004). Phytochemicals from natural sources are simpler and are much accepted in the development of new 'Rasayana' drugs from plants. Biologically active compounds from natural source have gained good status in treating several ailments not only in humans but also in animals.

Detailed research on the chemistry and pharmacology of remedial products of plant origin are much essential and this may ultimately lead to the innovation of medicine that can be used in the treatment of several hazardous diseases (Samy *et al*., 2008).

Several plants like *Oxalis corniculata* as an anti-inflammatory (Sakat *et al*., 2010) *Dorstenia barteri* as an anti arthritic (Omisore *et al*., 2004), *Paeonia lactiflora* as an antiarthritic (Liu *et al*., 2005) and so many others has been reported for their significant pharmacological

Fig. 1(a-l): Pictures of *Stephania glabra*, *Woodfordia fruticosa* and *Cissampelos pareira* their distilled water extract and solubility testing distilled water extract of Woodfordia *fruticosa*, (a) *Stephania glabra*, (b) *Woodfordia fruticosa*, (c) *Cissampelos pareira*, (d) Distilled water extract of *Stephania glabra*, (e) Distilled water extract of *Woodfordia fruticosa*, (f) Distilled water extract of *Cissampelos pareira*, (g) Volatile oil accumulation in clevenger apparatus, (h) Pictures showing insolubility of dry distilled water *Woodfordia fruticosa* extract in ethanol (s_1) distilled water (s_2) Methanol (s_3) Chloroform, (i) (s_4) n-hexane (s_5) Ethyl acetate, (j) (s_6) Pet ether (s_7), (k) Solubility testing for *Woodfordia fruticosa* dry distilled water extract after dissolving in chloral hydrate, (a_1) Solution ethanol, (a_2) n-hexane, (a_3) Chloroform, (a_4) Distilled water, (a_5) Ethyl acetate, (a_6) Pet ether

activities (Karim *et al.*, 2011; Sohail and Sohail, 2011; Sohail *et al.*, 2011). Similarly, Numbers of pharmacological activities have already been reported for *Stephania glabra*, *Woodfordia fruticosa* and *Cissempelos pareira*.

Stephania glabra (Fig. 1a) is a large climbing shrub with greenish yellowish flowers and large tubers up to 30 kg. It grows at subtropical and temperate Himalayas from an altitude of 7000 ft from sea level from Sindh eastward and Khasia hills and Pegu (Chopra *et al.*, 1994).

Woodfordia fruticosa (Fig. 1b) (Lythraceae) is much branched, semi deciduous, undershrub or shrub up to 1-3 m high, rarely upto 3 m. Plant is found throughout in India, ascending to 1500 m in Himalaya and also in the gangestic plains. Plant is known as Dhataki in Sanskrit, fire flame bush in English and dhai in Hindi. The brhat gangadhara churna is an important formulation in the dose of 3-6 g which has been specified in Ayurvedic pharmacopoeia (Khandelwal and Kokate, 1996). *Woodfordia fruticosa* is an important ingredient of several Ayurvedic formulations. According to Ayurveda, 'Rasayana' from the plant, is used as an Atisara, Raktapitta, Trsna, varna, and visarpa (The Ayurvedic Pharmacopoeia of India). *Cissempelos pareira* (Menispermaceae) is a climbing shrub, 2-5 m high with a thickened root. The leaves of the plant are orbicular in shape with 7.14 cm diameter. Leaves are membranous veined and glabrous.

Flowers are green, male ones in spikes, 7-10 cm long, with a little round leaflet at the base of every flower. *Cissempelos pareira* (Fig. 1c) Linn. is a perennial, deciduous climber commonly present throughout sub topical and topical region of India up an altitude of 2000 m. In Indian 'Ayurveda' *Cissempelos pareira* is known as Laghupatta (Chopra *et al.*, 1994).

By considering the importance of these plants in 'Ayurvedic' medicinal system the present study is focused on their pharmacognostical evaluation.

MATERIALS AND METHODS

Plant material and chemicals: The plant material (leaves of *Woodfordia fruticosa*, *Cissampelos pareira* and rhizome of *Stephania glabra*) was collected from 'Gharsi' village hills of Solan district of Himachal Pradesh, India. All plants were authenticated at department of forestry Dr. Y.S. Parmar University Solan, India. All the plant were linked to UHF-Herbarium with field book number 12544, 12545 and 12546 for *Cissampelos pareira*, *Woodfordia fruticosa* and *Stephania glabra*, respectively.

Physicochemical evaluation: To determine the different ash values and extractive values of each powdered drug, procedures from Herbal and Ayurvedic pharmacopoeia were followed.

Determination of ash values: Determination of ash values are meant for detecting low-grade products, exhausted, sandy or earthy matter and inorganic matters. It can also be utilized to detect the chemical constituents by using water soluble ash and acid insoluble ash.

Total ash: Accurately about 3 g of air dried powder of leaves of WFE, CPE and SGE were weighed separately in a tarred silica crucible and incinerated at a temperature not exceeding 450°C until free from carbon. Crucible was then cooled. Obtained ash was weighed and the percentage of total ash with reference to the air dried powdered drug was calculated.

Acid insoluble ash: Half amount of ash from total ash was boiled for 5 min with 25 mL of dilute HCl. The residue was collected on ash less filter paper and washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated.

Water soluble ash: The second half of total ash was boiled for 5 min with 25 mL of water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the

Table 1: Calculated percentage yield of ash and extractive values from dried powder of leaves of *Woodfordia fruticosa* (WFE), *Cissempelos pareira* (CPE) and rhizomes of *Stephania glabra* (SGE)

	Value (%)		
Test name	WF	CP	SG
Ash value			
Total ash	7.69	8.26	16.3
Water soluble	4.99	3.72	7.28
Acid insoluble	1.41	1.26	3.88
Extractive value			
Water soluble	10.25	12.93	9.62
Alcohol soluble	5.05	11.5	8.16

weight of the ash. The difference in weights represents the water-soluble ash. The percentage of water-soluble ash was calculated. The results are presented in Table 1.

Extractive value

Alcohol soluble extractive value: About 5 g of coarsely powdered air-dried drug was macerated with 100 mL of alcohol in a closed flask for 24 h. The flask was shaken frequently for 6 h and allowed to stand for 18 h. It was then filtered rapidly taking precaution against loss of alcohol. The filtrate (25 mL) was evaporated to dryness in tarred flat-bottomed shallow dish, dried at 105°C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

Water soluble extractive value: Coarsely powdered air-dried drug (5 g) was macerated with 100 mL of chloroform water in a closed flask for 24 h. The flask was shaken frequently for 6 h and allowed to stand for 18 h. It was then filtered rapidly taking precautions against loss of chloroform water. Twenty five milliliter of the filtrate was evaporated to dryness in tarred flat-bottomed dish dried at 105°C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

Extraction procedure

Ethanolic extraction by soxhlet apparatus: The collected, cleaned and powdered leaves and rhizome were taken for the extraction purpose. About 160 g of powdered leaves of WFE and CPE and rhizome of SGE were evenly packed in separate soxhlet apparatus. Ethanol (95%) and petroleum ether were used as solvent. The solvents used were purified before use. The extraction was carried out by hot continuous extraction for about 20 h. After extraction, the extract was filtered while hot through Whatman filter paper. The extract was concentrated by vacuum distillation to reduce the volume to 1/10. The concentrated extract was transferred to 100 mL beaker and the remaining solvent was evaporated on the water bath. Extracts so obtained were collected and placed in desiccator to remove the excessive moisture. The dried extracts were packed in air tight container and used for further studies such as phytochemical screening.

Distilled water extraction by maceration: Distilled water extraction was done by maceration for 72 h. Obtained extract (Fig. 1d-f) were vacuum filtered and evaporated on water bath. The dried extracts so obtained were sticky and insoluble. For this reason liquid extracts before evaporation were used for their phytochemical screening.

Phytochemical evaluation: Khandelwal and Kokate (1996) and Farnsworth (1966) detailed Phytochemical testing was performed to identify presence or absence of different phytoconstituents.

Hydrodistillation: About 30 g of powdered leaves of CPE was added to Clevenger apparatus (for oil lighter than water).The extraction was done for 9 h and amount of oil obtained was measured. Percentage yield of volatile oil was calculated.

Chromatography: TLC plate of silica gel 'G' were prepared by using pouring method. The plates were kept for activation in oven.

Solvent system:

- Ethyl acetate: Toluene (7:93)
- Hexane: Chloroform: Methanol (8:2:1)

Visualizing agent: Iodine chamber.

RESULTS

Total ash values were found to be 7.69, 8.26 and 16.3% for WFE, CPE and SGE, respectively. Water soluble ash was found to be 4.99, 3.72 and 7.28% for WFE, CPE and SGE, respectively. Acid insoluble ash for WFE was reported as 1.41%. Acid insoluble ash for CPE and SGE were found as 1.26 and 3.88%. The water soluble extractive value for WFE was found to be 10.25% and for CPE and SGE were reported as 12.93 and 9.62%. The alcohol soluble extractive values were reported as 5.05, 11.5 and 8.16% for WFE, CPE and SGE, respectively. Alkaloids, tannins, flavonoids, carbohydrates and phenols were found to be present in the ethanolic extract of CPE. Alkaloids, flavonoids and carbohydrates found in petroleum ether extract whereas alkaloids, carbohydrates, tannins were found in distilled water extract of CPE.

The ethanolic extract of SGE was found to be positive in amino acids, alkaloids, flavonoids, carbohydrates, phenols and saponin. Amino acid, flavonoids, carbohydrates and phenols were found to be present in petroleum ether extract of the plant. Water extract of the SGE showed positive results for flavonoids, carbohydrates and saponin.

DISCUSSION

The present study is an attempt to evaluate physicochemical and phytochemical aspects in the leaves of WFE, CPE and in the rhizome of SGE.

However, physicochemical and phytochemical evaluation of flowers WFE (Priyatno *et al.*, 2011) and leaves of CPE (Hemraj *et al.*, 2012a) has been already reported (Das *et al.*, 2007).

Phytochemical evaluation (Table 2): The result obtained for phytochemical screening from present study was compared with the previous reported phytochemical studies on these plants. From the leaves and flowers of *Woodfordia fruticosa*, phytoconstituents like tannins, flavanoids, anthraquinone glycosides and polyphenols has been reported (Das *et al.*, 2007). Along with these saponin glycoside and carbohydrates were newly reported in the present study.

In earlier investigations roots of *Cissempelos parera* has found to contain 0.33% of alkaloids, mainly hyatin and bebeerine, 0.2% essential oils, 3.4% fixed oils and a sterol (Ramirez *et al.*, 2003).

In present study tannins, flavonoids, carbohydrates and phenols were newly reported in the extracts of CPE.

Table 2: Phytochemical evaluation of ethanolic, petroleum ether and distilled water extract of *Woodfordia fruticosa* (WFE), *Cissempelos pareira* (CPE) and *Stephania glabra* (SGE)

	Ethanolic			Petroleum ether			Distilled water		
Name of test	WF	CP	SG	WF	CP	SG	WF	CP	SG
Amino acid	-	-	+	-	-	+	-	-	-
Anthraquinone	+	-	-	-	-	-	-	-	-
Alkaloids	-	+	+	+	+	-	-	-	-
Tannins	+	+	-	+	-	-	+	+	-
Flavonoids	+	+	+	+	+	+	+	+	+
CHB*	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	-	+	-	-	-
Saponin	+	-	+	-	-	-	+	-	+
Fats and oil	-	-	-	-	-	-	-	-	-

*Carbohydrates, +: Present, -: Absent

From the ethanolic extract of *Stephania glabra* four alkaloid namely palmatine, palmatine, dehydrocorydalmine and stepharanine has been isolated along with a novel morphine like alkaloid named 'Gindarudine' (Hemraj *et al.*, 2012b). In present study, amino acids, alkaloids, flavonoids, carbohydrates, phenols and saponin, Amino acid, flavonoids, carbohydrates and phenols were reported.

Physicochemical evaluation ash values and phytochemical screening of all three plants were performed as per the standard procedure (Table 1). The obtained total ash value of WFE was found to be within the standard limit which is 10%. The water soluble ash and acid insoluble ash value was found to exist within the standard limit of more than 2 and 1%, respectively. Total ash values for CPE and SGE was found to be 8.26 and 16.3%, respectively. Water soluble ash, acid insoluble ash values for CPE and SGE were calculated and compared with their standard values (Table 1).

The water soluble extractive values and alcohol soluble extractive value for WFE, CPE and SGE were reported (Table 1).

Hydrodistillation: Hydrodistillation of dried leaves powder of CPE was performed which provided 0.33% v/w of volatile oil from 30 g of powdered material. Same amount of volatile oil has already been reported for WFE and SGE in a work (Hemraj *et al.*, 2012a) (Fig. 1g).

Solubility testing: Solubility of sticky distilled water extracts were checked in different solvents. The extracts were found to be insoluble in ethanol, methanol, petroleum ether, n-hexane, distilled water, chloroform, ethyl acetate (Fig. $1s_1$-s_7). Chloral hydrate was tried as solvent. All the extracts were found to be soluble in chloral hydrate. The extract treated with chloral hydrate solution was again checked for solubility. Extracts were dissolved in chloroform, distilled water this time on stirring with glass rod (Fig. $1a_1$-a_6).

R_f values: R_f values from distilled water extract were obtained from two different polarity solvent systems Toluene: Ethyl acetate in the ratio of 93:7 and in Hexane: Chloroform: Methanol in the ratio of 8:2:1. Solvents were selected on the basis of polarity.

The R_f value for SGE (Fig. 2b), for WFE (Fig. 2a) and for CPE (Fig. 2c) in solvent system Toluene: Ethyl acetate were calculated. However the R_f value in Toluene: Ethyl acetate for SGE has already been reported as 0.93, 0.05 and 0.03 (Patwardhan *et al.*, 2010; Hemraj *et al.*, 2012b).

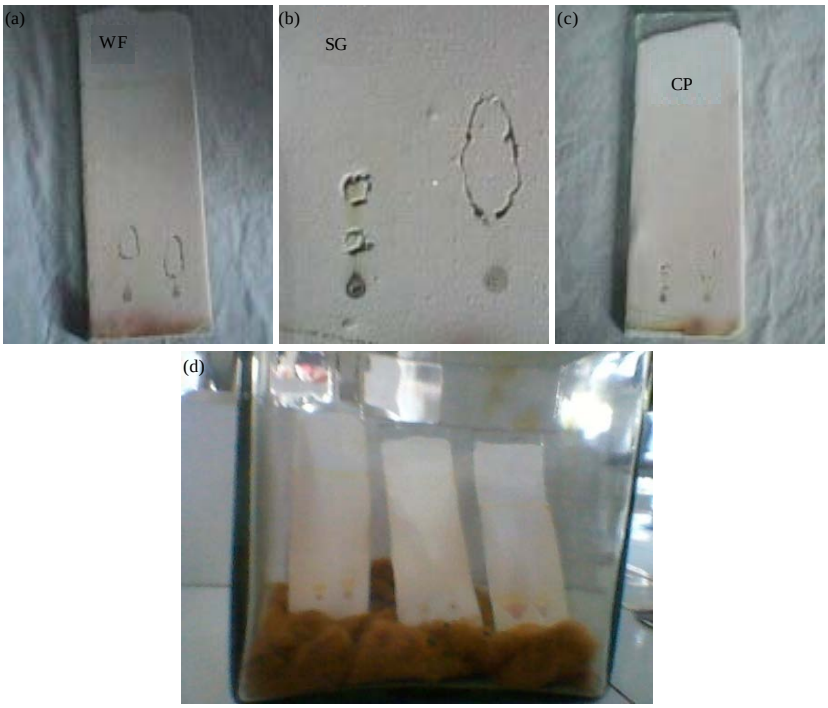

Fig. 2(a-d): TLC plates after being eluted in solvent system and being kept in Iodine chamber, a-d showing TLC plates 2 min after removing from iodine chamber, (a) *Woodfordia fruticosa*, (b) *Stephania glabra* and (c) *Cissampelos pareira*, (d) TLC plates of *Woodfordia fruticosa*, *Cissampelos pareira* and *Stephania glabra* in iodine chamber

Similarly, R_f values in TLC plates from the second solvent system (Hexane: Chloroform: Methanol) for CPE (Fig. 2d) for WFE were reported.

CONCLUSION

The plant *Woodfordia fruticosa*, *Cissempelos pareira*, *Stephania glabra* was taken up for the study to screen the physicochemical and preliminary phytochemical evaluation, TLC. *Cissempelos pareira* was studied for the determination of its volatile oil. The present investigations may help the researchers to evaluate and to find out new properties on these plants. The results of the study could be useful for the identification and preparation of a monograph of these plants.

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REFERENCES

Akerele, O., 1993. Nature's medicinal bounty: Don't throw it away. World Health Forum, 14: 390-395.

Baker, D.D. and K.A. Alvi, 2004. Small-molecule natural products: New structures, new activities. Curr. Opin. Biotechnol., 15: 576-583.

Chopra, I.C., K.L. Handa and L.D. Kapur, 1994. Chopra's Indigenous Drugs of India. 2nd Edn., Academic Publishers, Kolkata, India.

Das, P.K., S. Goswami, A. Chinniah, N. Panda, S. Banerjee, N.P. Sahu and B. Achari, 2007. *Woodfordia fruticosa*: Traditional uses and recent findings. J. Ethnopharmacol., 110: 189-199.

Farnsworth, R.N., 1966. Biological and phytochemical screening of plants. J. Pharm. Sci., 55: 225-276.

Hemraj, A. Gupta, A. Thakur and N. Upmanyu, 2012a. Hydrodistillation of *Stephania glabra* tubers and *Woodfordia fruticosa* leaves. Asian J. Pharm. Clin. Res., 5: 105-107.

Hemraj, N. Upmanyu, A. Gupta, A. Jindal and S. Jalhan, 2012b. Pharmacological activities of *Stephania glabra*, *Woodfordia fruticosa*, *Cissampelos pareira*: A review. Int. J. Pharmacy Pharm. Sci., 4: 16-23.

Karim, A., M.N. Sohail, S. Munir and S. Sattar, 2011. Pharmacology and phytochemistry of Pakistani herbs and herbal drugs used for treatment of diabetes. Int. J. Pharmacol., 7: 419-439.

Khandelwal, K.R. and C.K. Kokate, 1996. Practical Pharmacognosy Technique and Experiments. 3rd Edn., Nirali Prakashan, Pune, India, pp: 1-45.

Liu, Z.Q., H. Zhou, L. Liu, Z.H. Jiang and Y.F. Wong *et al.*, 2005. Influence of co-administrated sinomenine on pharmacokinetic fate of paeoniflorin in unrestrained conscious rats. J. Ethnopharmacol., 99: 61-67.

Omisore, N.O., C.O. Adewunmi, E.O. Iwalewa, B.T. Ngadjui, J. Watchueng, B.M. Abegaz and J.A. Ojewole, 2004. Antinociceptive and anti-inflammatory effects of *Dorstenia barteri* (Moraceae) leaf and twig extracts in mice. J. Ethnopharmacol., 95: 7-12.

Patwardhan, S.K., K.S. Bodas and S.S. Gundewar, 2010. Coping with arthritis using safer herbal options. Int. J. Pharm. Pharm. Sci., 2: 1-11.

Priyatno, L.H.A., E.Y. Sukandar, S. Ibrahim and I.K. Adnyana, 2011. Antihyperuricemic effect of ethanol extract of snake fruit (*Salacca edulis* Reinw.) Var. Bongkok on Wistar male rat. Proceedings of the 12th ASEAN Food Conference, June 16-18, 2011, BITEC Bangna, Bangkok, Thailand, pp: 108-114.

Ramirez, I., A. Carabot, P. Melendez, J. Carmona and M. Jimenez *et al.*, 2003. Cissampeloflavone, a chalcone-flavone dimer from *Cissampelos pareira*. Phytochemistry, 64: 645-647.

Sakat, S.S., A.R. Juvekar and M.N. Gambhire, 2010. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int. J. Pharm. Pharm. Sci., 2: 146-155.

Samy, R.P., P.N. Pushparaj and P. Gopalakrishnakone, 2008. A compilation of bioactive compounds from ayurveda. Bioinformation, 3: 100-110.

Sohail, F. and M.N. Sohail, 2011. *Silybum marianum* L. despite of its weedy nature is an important medicinal plant of NWFP, Pakistan. Int. J. Pharmacol., 7: 747-748.

Sohail, M.N., F. Rasul, A. Karim, U. Kanwal and I.H. Attitalla, 2011. Plant as a source of natural antiviral agents. Asian J. Anim. Vet. Adv., 6: 1125-1152.