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Antioxidant Potential of Himalayan Medicinal Plants *Angelica glauca, Alysicarpus vaginalis* and *Peristrophe bicalyculata*

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A B S T R A C T

The petroleum ether, chloroform, methanol and aqueous extract of *Angelica glauca* Edgew, *Alysicarpus vaginalis* (L.) DC and *Peristrophe bicalyculata* (Retz.) Nees were screened for their free radical scavenging properties using ascorbic acid, BHA and rutin as standard antioxidant. Free radical scavenging activity was evaluated using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical. The overall strongest antioxidant activity was found in methanolic extract for all the plants, The methanolic extract of *A. glauca* %inhibition of DPPH radical is up to 95.81%, *A. vaginalis* %inhibition of DPPH radical is up to 90.36% and *P. bicalyculata* % inhibition of DPPH radical is up to 86.33%. The IC_{50} values of the extracts ranged between 69.42 to 475.36 µg/ml and standard antioxidant levels varied from 21.49 to 157.63 µg/ml. The present study reveals that the selected plants would exert several beneficial effects by virtue of their antioxidant activity and could be harnessed as drug formulation. These plants may be used as a source of strong natural antioxidants.

Introduction

An antioxidant is a molecule that inhibits the oxidation of other molecules. The definition of antioxidants, given in 1995 by Halliwell and Gutteridge, stated that an antioxidant is "any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate" (Halliwell and Gutteridge, 1995). In 2007, Halliwell gave a more specific definition, stating that an antioxidant is "any substance that delays, prevents or removes oxidative damage to a target molecule" (Halliwell, 2007). Our body is rich in endogenous antioxidants, the substances that have the ability to stop free radicals formation or to limit the damage they cause (Tomas *et al.*, 1997). The effectiveness of current used exogenous antioxidants arises most probably from the increase of the endogenous free radical scavengers as enzymes (superoxide dismutase and selenium-dependent glutathione peroxidase), vitamins (alpha tocopherol and ascorbic acid). Many plants have also been found to possess free radical scavenging activity (polyphenols, alkaloids and terpenoids). Low levels of one or more of the essential antioxidants have been shown to be associated with many disorders including cancer, inflammation, atherosclerosis,

coronary heart disease and diabetes. Many medicinal herbs contain antioxidant compounds which protects the cells against the damaging effects of reactive oxygen species.

Angelica glauca genus *Angelica* (family: Apiaceae) is recognised globally for its uses in traditional and modern system of medicine. The estimated 110–115 species of the genus worldwide, 87 species occur in Asia (Pimenov and Leonov, 2004). Three species namely *Angelica glauca* Edgew, *A. archangelica* L. and *A. nubigena* Cl. are reported from the Indian Himalaya (Samant *et al.,* 1998). *A. glauca*, locally called as Choru or Gandravan, being native and endemic of the Himalayan region, is distributed along 2000 to 3,800m in Uttarakhand, Jammu and Kashmir and Himachal Pradesh (Butola and Badola, 2004). The species is well known for its aromatic as well as medicinal values.

Alysicarpus vaginalis genus *Alysicarpus* belongs to family Papilionaceae. There are approximately 78 species in their genus with 20 species are reported in India (Haines, 1978). They commonly found in open grass land, crop fields, way sides, to 1000m Garhwal Himalaya, almost throughout India, ascending to 1000m Afghanistan, Pakistan and tropical America.

The genus comprises annual Prostrate Herbs, perennial. Several species of *Alysicarpus* has been used in indigenous system of medicine an anti-inflammatory in stomachache, and also an antidote to snake bite. It is also used in skin diseases and as a diuretic. The leaves are used in fever and jaundice (Shankarnarayan, 1993).

Peristrophe bicalyculata genus *Peristrophe* belongs to family Acanthaceae, is an erect herbs, stem slender, branched 30-70cm height found in forest undergrowth, hedges and waste band almost throughout India and in Garhwal Himalaya it is found at an altitude of 500 to 1,400m. The leaves of the plant were used traditionally as analgesic, antipyretic, anti-inflammatory, sedative, stomachic, anticancer, fertility, diuretics and diarrhea. *P. bicalyculata* is used by the traditional healers for curing many skin related problems; as an antidote for snake poison when macerated in an infusion of rice, and as an insect repellant (Abdulazeez *et al.,* 2013). Paste of *P. bicalyculata* applied on wound, flowers use as source of *bee-forage*. The essential oil shows tuberculostatic activity *in vitro*. It inhibits the growth of various strains of *Mycobacterium tuberculosis*. Ayurvedic Pharmacopoeia of India recommends the dried root in insomnia and for fear-psychosis in children (Khare, 2007).

Materials and Methods

Chemicals

2,2-diphenyl-1-picryl-hydrazyl (DPPH), potassium persulfate, 2,2'-azinobis-(3 ethylbenzothiozoline-6-sulfonic acid)-diammonium salt (ABTS), 6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), Rutin, sodium nitroprusside, ferrozine, ferrous chloride, ethylenediamine tetracetic acid (EDTA) disodium salt, Butylated Hydroxyl Anisole (BHA) and ascorbic acid were obtained from Himedia Chemicals or Sigma. All other reagents used were of analytical grade.

Collection and identification of plants

The plants included in this study involve *Angelica glauca* (Stem and Root) collected from Tungnath- Chandrashilla and Track of Rudaranath (Rudraprayag), *Alysicarpus vaginalis* (Root) from Chauras and Kirtinagar (Tehri Garhwal) and *Peristrophe bicalyculata* (Leaves) collected from Chauras, Kirtinagar

(Tehri Garhwal), Srinagar, Srikot and Pauri (Pauri Garhwal) Uttarakhand (Fig. 1). Plant were authenticated at Garhwal University Herbarium (GUH), a herbarium voucher specimen were submitted.

Preparation of extract

Collected plant materials was properly washed with water, dried under shade at room temperature and crushed to small pieces by using pestle and motor. The plant extracts were prepared by immersing 200g of powdered plant material in 600ml of four different solvents according to polarity low to high i.e. petroleum ether (PET), chloroform (CHF), methanol (MeOH) and aqueous (H_2O), loaded in Soxhlet assembly and extracted for 72 h through successive method (Ahmed *et al.*, 1998). Plant extracts were filtered through Whatman No. 1 filter paper and crude extracts obtained by removing solvent in vacuum evaporator at 30°C. Residues were stored at 4°C until further use.

Evaluation of antioxidant activity

DPPH free radical scavenging activity

DPPH (2, 2-diphenyl picryl hydrazyl) is a commercially available stable free radical, which is purple in colour. The antioxidant molecules present in the herbal extracts, when incubated, react with DPPH and convert it into di-phenyl hydrazine, which is yellow in colour. The degree of discoloration of purple to yellow was measured at 517nm, which is a measure of scavenging potential of plant extracts.

A 2ml aliquot of solution was added to 2ml of 2×10^{-4} mol/L ethanolic DPPH solution. The mixture was shaken vigorously and the absorbance was measured at 517nm immediately. The decrease in absorbance was determined at 15 and 30min until the absorbance reached a steady state (after nearly 30 minute). The DPPH with corresponding solvents (without plant material) serves as the positive control. The respective solvent of plant extracts (without DPPH) serves as blank (Sheng *et al.*, 2011). All the tests were performed in triplicate and the DPPH radical scavenging activity of the plant extract was calculated as the percentage inhibition according to the formula:

$$\% \text{ Inhibition of DPPH free radical} = \left[\frac{(A_{blank} - A_{sample})}{A_{blank}}\right] \times 100$$

Results and Discussion

DPPH free radical scavenging ability

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical changes its colour from purple to yellow in the presence of antioxidant and is most widely used to evaluate the antioxidant potential of extracts. The method is based on the hydrogen donating capability of the extract which scavenges DPPH free radical. Scavenging of the DPPH radical is also linked to the inhibition of lipid peroxidation.

The results of the assay demonstrated antioxidant activity of *Angelica glauca* extracts suggesting that the extracts are capable of donating hydrogen and acting as natural antioxidants. The methanolic extract of *A. glauca* (Fig. 4) was potent in scavenging DPPH radical in comparison to petroleum ether (Fig. 2), chloroform (Fig. 3) and aqueous extract (Fig. 5). The methanolic extract of *A. glauca* % inhibition of DPPH radical is up to 95.81%. The radical scavenging ability was significantly low when compared to synthetic antioxidants like BHA and ascorbic acid. The scavenging ability of the methanol extract was however comparable to rutin. The potential to scavenge DPPH radical was measured by determining IC_{50} value which indicate the concentration

required to inhibit 50% of DPPH free radicals. Lower values of IC_{50} indicate higher potency to scavenging DPPH free radicals of plants extract. IC_{50} value of the methanolic extract (69.42 µg/ml) was much lower in comparison to chloroform extract (100.71 µg/ml), petroleum ether extract (261.35 µg/ml) and water extract (231.65 µg/ml) of *A. glauca* (Fig 15). The IC_{50} value of methanolic extract (69.42 µg/ml) and chloroform extract (100.71 µg/ml) of *A. glauca* is much lower in comparable to standard synthetic antioxidant BHA (157.63 µg/ml) but higher than the other synthetic antioxidant like rutin (45.19 µg/ml) and ascorbic acid (21.43 µg/ml) (Fig. 15).

The methanol extract of *A. vaginalis* (Fig. 8) was potent in scavenging DPPH radical in comparison to chloroform (Fig. 7), petroleum ether (Fig. 6) and aqueous extract (Fig. 9). The methanolic extract of *A. vaginalis* % inhibition of DPPH radical is up to 90.36%. The IC_{50} value of methanol extract was (144.92 µg/ml), chloroform extract (243.64 µg/ml), petroleum ether extract (475.36 µg/ml) and aqueous (371.17 µg/ml) (Fig. 15). The IC_{50} value of methanolic extract (144.92 µg/ml) is lower than synthetic antioxidant BHA (157.63 µg/ml) (Fig. 14).

The methanol extract of *P. bicalyculata* (Fig. 12) exhibited maximum potency in scavenging DPPH radical in comparison to petroleum ether (Fig. 10), chloroform (Fig. 11) and aqueous extract (Fig. 13). The methanolic extract of *P. bicalyculata* % inhibition of DPPH radical is up to 86.33% it's lower than other two medicinal plants. IC_{50} value of the methanolic extract (153.79 µg/ml) was much lower than that of chloroform (330.16 µg/ml), petroleum ether extract (243.79 µg/ml) and aqueous extract (272.26 µg/ml) (Fig. 15). IC_{50} value of methanolic extract of *P. bicalyculata* was comparable to BHA (157.79 µg/ml), however it was much higher than synthetic antioxidant rutin (45.19 µg/ml) and ascorbic acid (21.43 µg/ml) (Fig. 14). Lower value of IC_{50} of the extract showed the potent antioxidant activity presence in the extracts.

The best antioxidant activity was showed by methanolic extract of *A. glauca* in comparison to other two medicinal plants. Joshi *et al.*, (2008) reported antioxidant activity of water extract of *A. glauca*, the scavenging activity of the water extract ranged from 14.58% to 71.53% as the concentration increased 5 to 25 mg/ml. The extract exhibited moderate scavenging activity, which was less than the standards. But in the present observation water extract of *A. glauca* showed 50.94% to 69.36% inhibition in 100 to 400 µg/ml concentration. Irshad *et al.*, (2011) reported that the essential oil of *A. glauca* exhibited good DPPH radical scavenging activity showing (93.4% of inhibition and 45.05% inhibition of peroxidation).

Collection and identification of plants

Name of plants	**Family**	**Accession No.**
***Angelica glauca* Edgew.**	Apiaceae	**GUH 20748**
***Alysicarpus vaginalis* (L.) DC.**	Fabaceae	**GUH 20749**
***Peristrophe bicalyculata* (Retz.) Nees**	Acanthaceae	**GUH 20750**

Fig.1 Map of medicinal plants collection sites

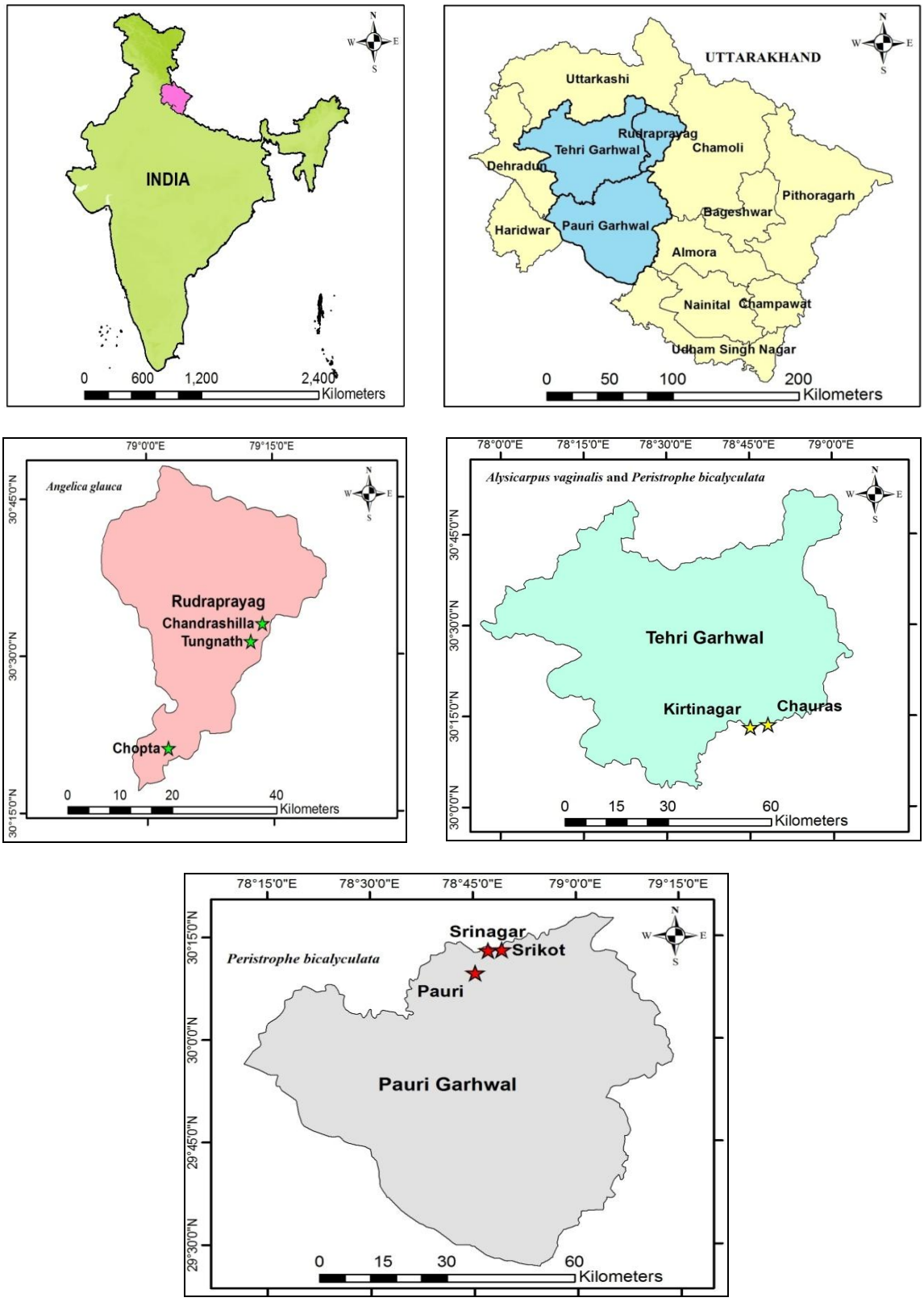


Fig.2 % Inhibition of DPPH free radicals by *A. glauca* petroleum ether extract

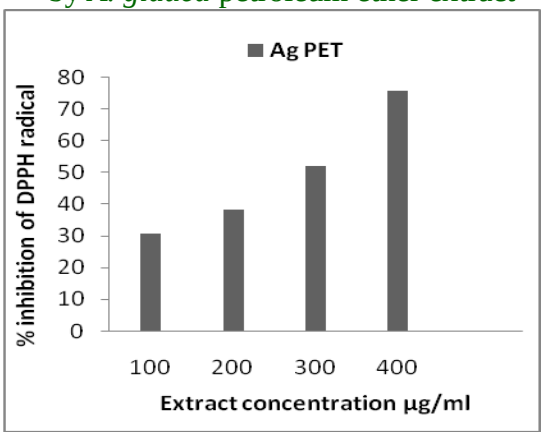


Fig.3 % Inhibition of DPPH free radicals by *A. glauca* chloroform extract

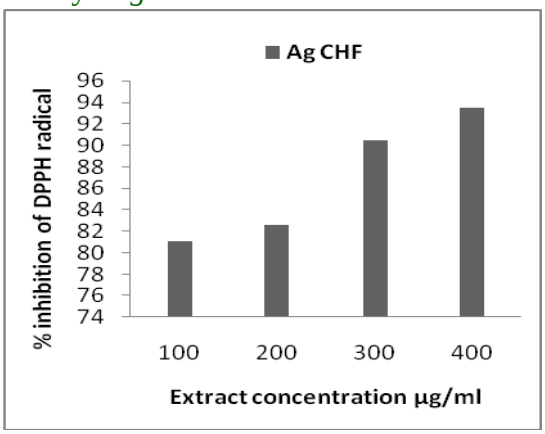


Fig.4 % Inhibition of DPPH free radicals by *A. glauca* methanol extract

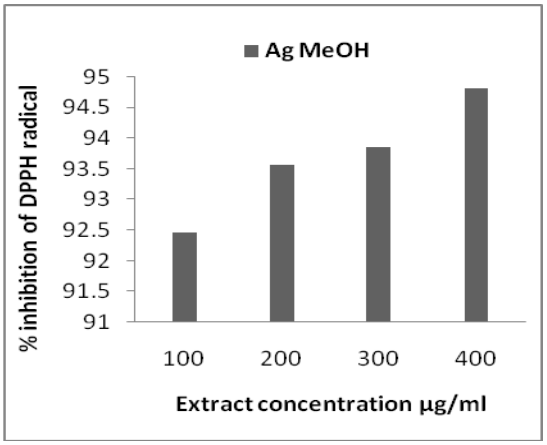


Fig.5 % Inhibition of DPPH free radicals by *A. glauca* aqueous extract

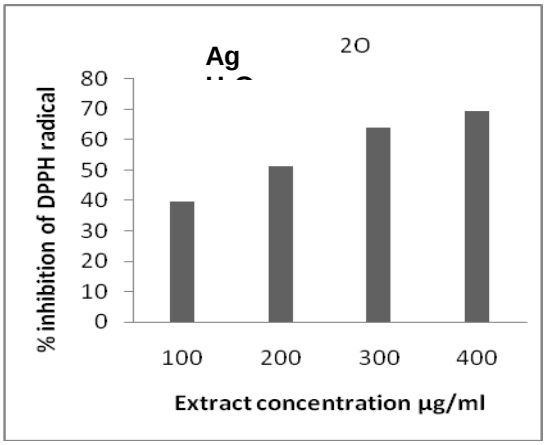


Fig.6 % Inhibition of DPPH free radicals by *A. vaginalis* petroleum ether extract

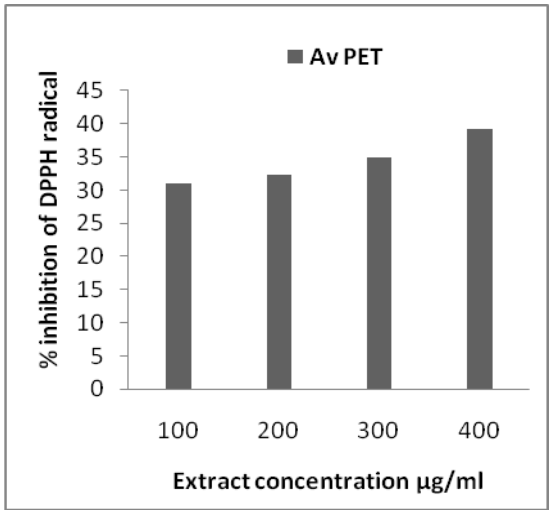


Fig.7 % Inhibition of DPPH free radicals by *A. vaginalis* chloroform extract

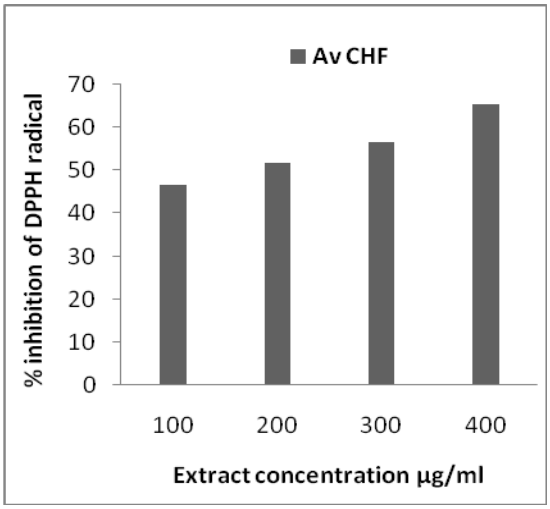


Fig.8 % Inhibition of DPPH free radicals by *A. vaginalis* methanol extract

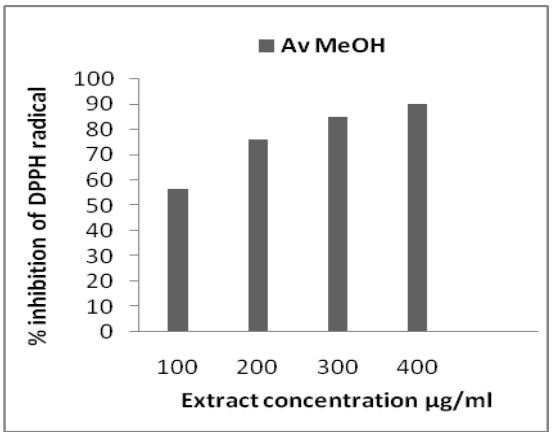


Fig.9 % Inhibition of DPPH free radicals by *A. vaginalis* aqueous extract

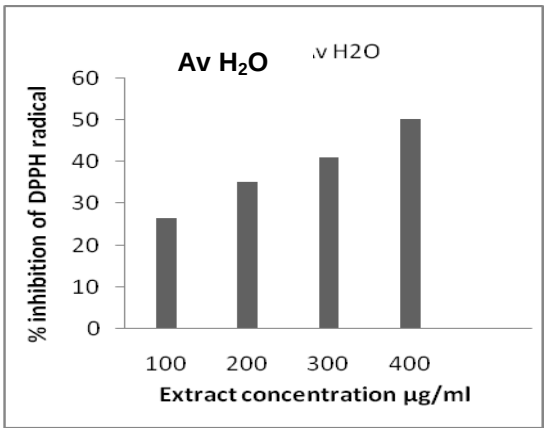


Fig.10 % Inhibition of DPPH free radicals by *P. bicalyculata* petroleum ether extract

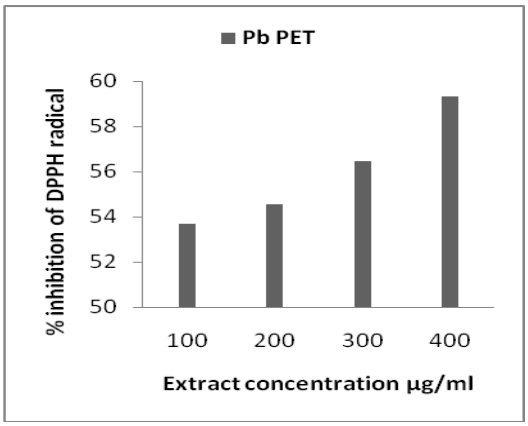


Fig.11 % Inhibition of DPPH free radicals by *P. bicalyculata* chloroform extract

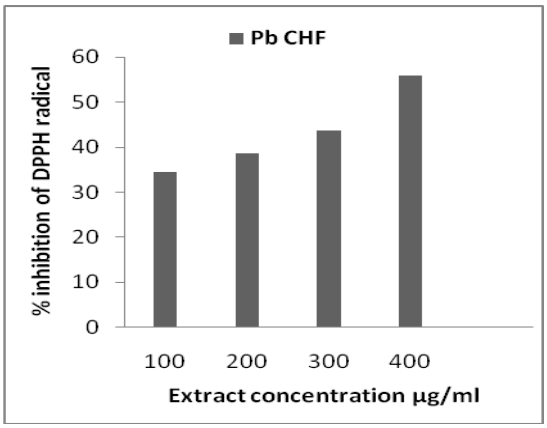


Fig.12 % Inhibition of DPPH free radicals by *P. bicalyculata* methanol extract

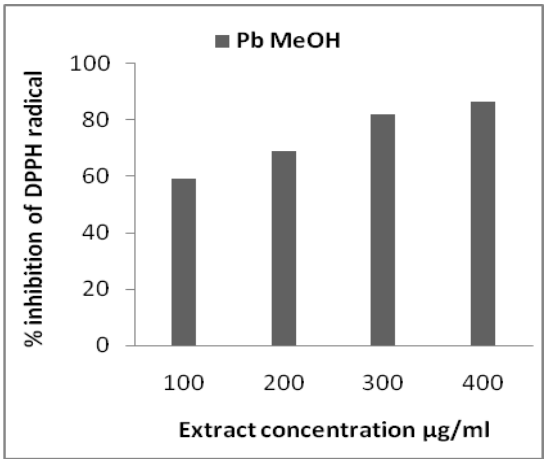


Fig.13 % Inhibition of DPPH free radicals by *P. bicalyculata* aqueous extract

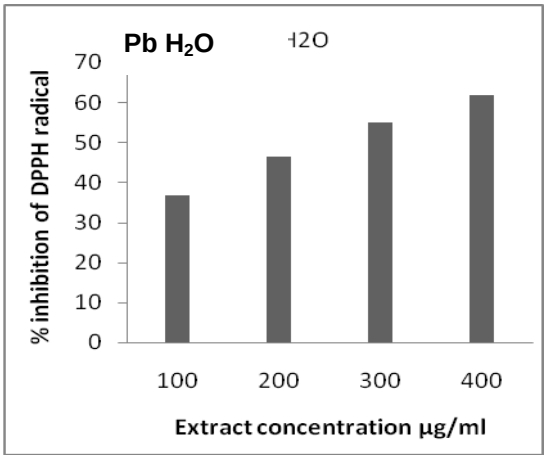


Fig.14 Comparison of the DPPH free radicals scavenging ability by methanolic extracts of *A. glauca*, *A. vaginalis* and *P. bicalyculata* with standard antioxidant.
BHA= Butylated Hydroxyl Anisole, Ag MeOH= Methanolic extract of *A. glauca*, Av MeOH= Methanolic extract of *A. vaginalis*, Pb MeOH= Methanolic extract of *P. bicalyculata*

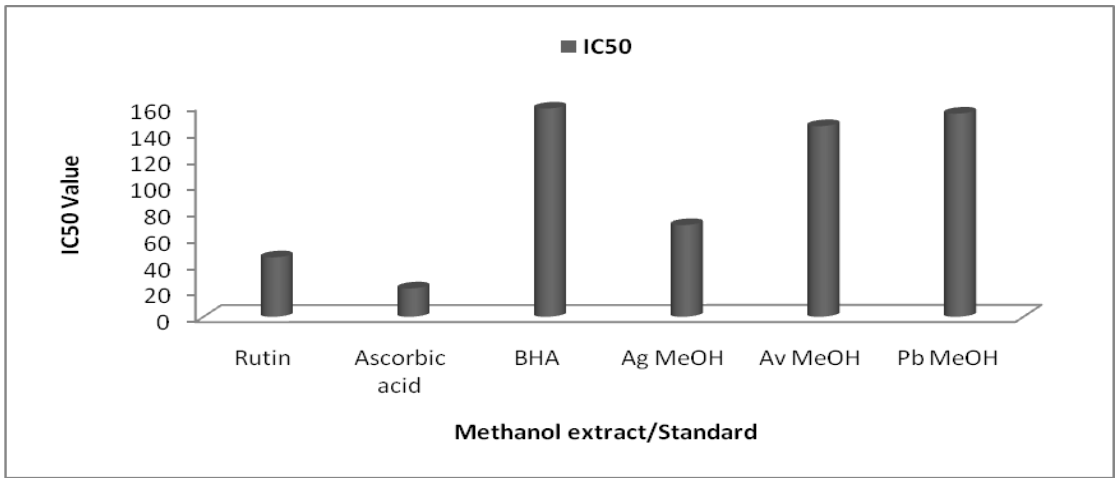
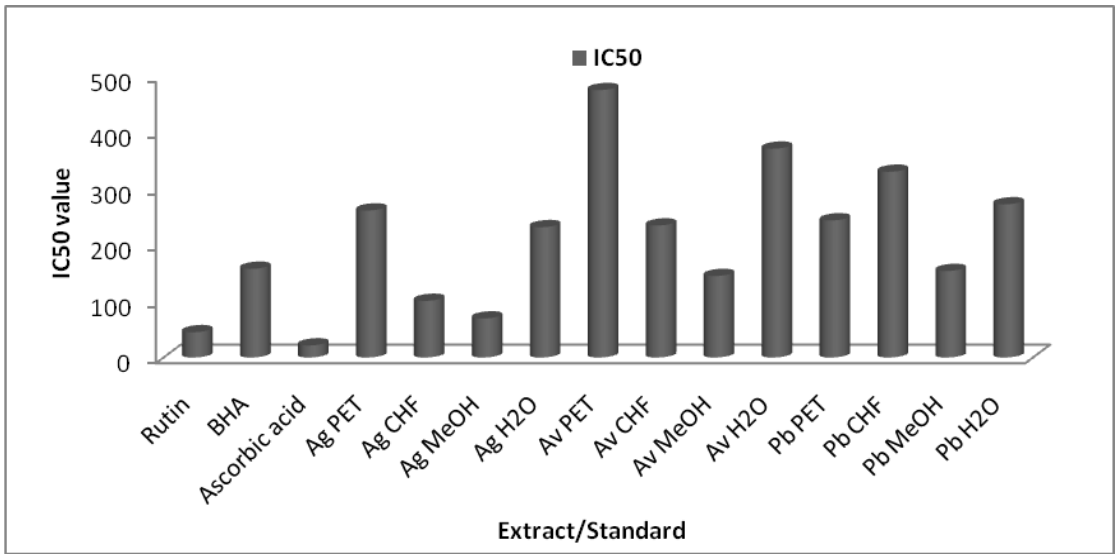


Fig.15 Comparison of the DPPH free radicals scavenging ability by various extracts of *A. glauca*, *A. vaginalis* and *P. bicalyculata* with standard antioxidant.



Note: BHA (butylated hydroxyl anisole), RUTIN, AA (ascorbic acid), DPPH (2, 2-diphenyl-1-picrylhydrazyl). Ag PET, Ag CHF, Ag MeOH, Ag H_2O is petroleum ether, chloroform, methanol and aqueous extracts of *A. glauca*. Av PET, Av CHF, Av MeOH, Av H_2O are petroleum ether, chloroform, methanolic and aqueous extracts of *A. vaginalis.* Pb PET, Pb CHF, Pb MeOH, Pb H_2O- petroleum ether, chloroform, methanolic and aqueous extracts of *P. bicalyculata* respectively.

On the basic of prior published report minimum work has been done on antibacterial and antioxidant activity of medicinal creeping annual herbs *A. vaginalis*. Narintorn *et al.*, (2014) reported the antioxidant activity of water and ethanol extract of *A. vaginalis*. The highest antioxidant activity was found in DPPH method by ethanolic extract of *A. vaginalis*. IC_{50} value of ethanolic extract was (345.70 µg/ml) and water extract (381.98

µg/ml). The lowest IC_{50} value indicates the highest potency of inhibition or scavenging. In the present study the IC_{50} value of aqueous extract of *A. vaginalis* is 371.17 µg/ml and methanolic extract is 144.92 µg/ml.

Johnley *et al.,* (2014) reported the antioxidant activity of whole plant of *P. bicalyculata* by DPPH method. They used the three solvent petroleum ether, methanol and ethyl acetate. The results showed highest antioxidant activity by methanol extract in 1000 µg/ml concentration (63.15%) followed by same concentration of petroleum ether (50.28%) and ethyl acetate (55.45%). The IC_{50} value of methanolic extract is (612 µg/ml), petroleum ether extract (1020 µg/ml) and ethyl acetate extract (830 µg/ml) in 1000 µg/ml concentration. Rutin used as standard give IC_{50} value (480 mg/ml) on methanol, petroleum ether and ethyl acetate. Another researcher work on antioxidant activity of water, ethanol and acetone extract of *P. bicalyculata* by DPPH method on 50 µg/ml concentration. The IC_{50} value of water extract is (471 µg/ml), ethanol (501 µg/ml) and acetone extract (144.7 µg/ml). The maximum scavenging percentage was found in acetone extract than ethanol and water extract of *P. bicalyculata*. Krishnamoorthy *et al.,* (2014) determine the antioxidant activity of water ethanol and acetone extract of *P. bicalyculata* by DPPH method on 50 mg/ml concentration.

In this paper we reported the data of antioxidant activities of different extracts of *A. glauca, A. vaginalis* and *P. bicalyculata*. The methanol extract of *A. glauca, A. vaginalis* and *P. bicalyculata* showed the highest value of total antioxidant capacity as compare to other extract. The methanol extract of *A. glauca* demonstrated significant reducing power in the concentration 100 to 400 µg/ml range between 90.43% and 94.81% of inhibition. The methanolic extract of *A. vaginalis* exhibited significant reducing power in the concentration range of 100−400 µg/ml and also the methanolic extract of *P. bicalyculata* exhibited highest reducing power than other extract. In all the plants extract the methanolic extract of *A. glauca* showed highest reducing power (95.81%), which was even better than other extract in the concentration range of 100−400 µg/ml. Minimum values of IC_{50} indicate maximum potency to scavenging DPPH free radicals of plants extract. IC_{50} value of the methanolic extract of *A. glauca* was 69.42 µg/ml, IC_{50} value of methanol extract of *A. vaginalis* was 144.92 µg/ml and IC_{50} value of the methanolic extract was 153.79 µg/ml. *P. bicalyculata* showed the potent DPPH free radical scavenging ability in the extracts which is better than the synthetic antioxidant BHA (157.63 µg/ml). The methanolic extract of *A. glauca* on 400 µg/ml concentration showed the maximum inhibition (95.81%) followed by *A. vaginalis* (90.36%) and *P. bicalyculata* (86.33%). The methanolic extract of *A. glauca, A. vaginalis* and *P. bicalyculata* showed effective antioxidant activity. In other words we can say these plants are used as a natural source of strong antioxidant.

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