Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2016, 5, 8:35-43

In vitro antioxidant activity and phytochemical screening of Garhwal Himalaya medicinal plants

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ABSTRACT

Bistorta macrophylla, B.vaccinifolia and Persicaria polystachya are used for the treatment and prevention of many ailments including tuberculosis, inflammation, pyretic, fever, flue, lungs disorders, diarrhea, vomiting, arthritis, gout, kidney stones or hyperacidity and hypertension. This study was aimed to evaluate the possible in vitro antioxidant activity and phytochemical screening of B. macrophylla, B.vaccinifolia and P. polystachya. The results of antioxidant activity study of B. macrophylla showed maximum activity in the methanolic extracts at different concentration of 20, 40, 60, 80 and 100 µg/ml. The percent inhibition of writhing response by the extract was 36.18%, 44.72%, 59.21%. 67.08% and 83.39% respectively. In the present work a potent anti-oxidant activity of methanolic extract of the whole plants of B. macrophylla were demonstrated, validating the ethno pharmacological claims. These experimental findings would further establish the scientific basis of the traditional uses of the plant in the management of different conditions as well as control of different disease.

Keywords: Antioxidant activity, Phenolic compounds and Phytochemical screening.

INTRODUCTION

Garhwal Himalayas are rich source of medicinal plants. These medical plants are used in recovering from various diseases. Medicinal plants represent a rich source of potent and powerful drugs. The treatment of human and animal disease depends mainly on natural products derived from plants, animals, microorganisms and minerals. *Bistorta macrophylla* also known as Kukhri belongs to the polygonaceae family and the root and leaves are used in traditional medicine. *B. macrophylla* has several medicinal values. In Bhutan, Tibetan and health care system of Uttarakhand, it is used as an antidiarrheal, antidysenteric, alleviates stomach pain, anti-inflammatory, anti-pyretic, used in lung disorders, associated with fever and flue. In Uttarakhand, the plant is used to treat cough, cold, tonsillitis and fever. The flowers are used to treat abdominal and back pain [1 & 2].

B.vaccinifolia (polygonaceae) family, commonly known as Inuri and masloon in Uttarakhand. *B.vaccinifolia* roots and leaves are used in different traditional medicine. The plant has several medicinal values. In Tibetan and health care system of Uttarakhand, it is used as an anti-inflammatory, anti-pyretic, used in lung disorders, associated with fever and flue, diarrhea and vomiting. In Nepal, the plant is used to treat cough, cold, Tonsillitis and fever. The flowers are used to treat abdominal and back pain. Leaves cooked as vegetable; roots decoction supposed to be useful in tuberculosis [3].

Persicaria polystachya (family Polygonaceae) and commonly known as saran. *P. polystachya* also contain oxalic acid (the distinctive lemony flavor of sorrel) – while it is not toxic this substance can bind up other minerals making them unavailable to the body and leading to mineral deficiency. *P. polystachya* leaves are nutritious and beneficial to eat in moderate quantities. Cooking the leaves will reduce their content of oxalic acid. People with a tendency to rheumatism, arthritis, gout, kidney stones or hyperacidity should take especial caution if including this plant in their diet since it can aggravate their condition. It's usually with scattered, numerous reddish glands, slightly fragrant [4].

Bistortia vaccinifolia

Persicaria polystachya

Bistorta macrophylla

MATERIALS AND METHODS

Collection and Identification

The materials included fresh and dry whole plant of *Bistortia vaccinifolia*, *Persicaria polystachya* and *Bistorta macrophylla* were collected from Tungnath (Chopta), Uttarakhand district, during July-August 2015. These plants were authenticated by the Taxonomy Laboratory, Department of Botany, HNB Garhwal University, Srinagar. The voucher specimens GUH 8631 (*B. vaccinifolia*), GUH 6683 (*P. polystachya)* and GUH 6894 (*B. macrophylla*) were deposited in the University herbarium for future records.

Preparation of plant Extract

The whole plants were first shade dried for a week. Then the crushed plant material were ground into coarse powder with the help of a mechanical grinder and soxhlet extracted with petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water using the soxhlet apparatus [5]. Each extract was evaporated to dryness under reduce pressure using a rotary evaporator. The extracts thus obtained were stored in air tight container at 4^{o}C until further analysis.

Chemicals

All the chemicals and reagents used were of analytical grade such as DPPH (2, 2-Diphenyl-1-picrylhydrazyl), sodium hydroxide, methanol, ethyl alcohol, hydrochloric acid and sulphuric acid (Merk India Ltd).

MATERIALS AND METHODS

Successive value

Accurately weighed 500gm coarse and air dried drug material were subjected to hot successive continuous extraction in soxhlet apparatus with different solvents with increase in polarity petroleum ether, benzene, chloroform, methanol, ethanol and finally with water. The extracts were filtered in each step concentrated and the solvent was removed by vacuum distillation. The extracts were dried in the vacuum dessicator and the residues were weighed [6]. Which contain maximum chemical compound are these categories as depend upon solvent nature and types.

Qualitative phytochemical analysis

The qualitative phytochemical analysis of all samples was carried out using standard methods. The extracts obtained as above are then subjected to qualitative tests for the identification of various plant chemical constituents. In addition, 50 gm of air dried or fresh plant material is also subjected to hydro-distillation to detect the presence of

volatile oil. The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents on the following lines [7].

Quantitative *phytochemical analysis*

The quantitative phytochemical analysis of all samples was carried out using standard methods. The extracts obtained as above are then subjected to quantitative tests for the identification of various plant chemical constituents. The plant material may be subjected to quantitative phytochemical analysis for the detection of various plant constituents' tannins [8], saponins [9], phenolic [10] and flavonoids [11] on the following lines.

Detection of chemical compound by TLC

Thin layer chromatography is a chromatography technique used to separate mixtures. TLC is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material usually silica gel G, aluminium oxide, or cellulose. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (mobile phase) is drawn up the plate via capillary action. TLC plates are prepared by spreading silica gel G on glass plate using distill water as solvent these plates are activated in oven at 110^{O}C for one hour. All extracts are applied separately and run in different solvent system of varying polarity. These plates are developed in Iodine chamber, UV chamber and spraying reagent for different spot of constituent chemical [12].

DPPH radical scavenging assay

The ability of the plant extract to scavenge DPPH free radicals was assessed by the standard method and adopted with suitable modifications. The stock solutions of extracts were prepared in methanol to achieve the concentration of 1 mg/ml. The dilutions were made to obtain concentrations of 20,40,60,80 and 100 µg/ml. The diluted solutions (1 ml each) were mixed with 2 ml of methanolic solution of DPPH in concentration of 1 mg/ml. After 30 min Incubation in darkness at room temperature (23^{o}C), the absorbance was recorded at 517 nm. The control sample contained all the reagents except the extract and the percentage inhibition was calculated using equation 1, whilst IC50 values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm [13].

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

The data are expressed as the mean ± SEM analyzed by one-way analysis of variance (ANOVA) and Tukey's t-test was used as the test of significance. P value<0.05 was considered as the minimum level of significance. All statistical tests were carried out using SPSS statistical software [14].

RESULTS AND DISCUSSION

Successive value of Polygonum Species:

The first step towards this goal is the antioxidant activity, TLC profile, successive value and phytochemical screening of Polygonum species (*B.vaccinifolia, P. polystachya*, and *B. macrophylla)*. The results of successive value, TLC profile, phytochemical screening and antioxidant activity as table 1, 2, 3 & 4 and fig. 1, 2, 3, 4 & 5.

Successive value:

B.vaccinifolia, P. polystachya, and *B. macrophylla* whole plants showed significant successive value are 4.21%, 3.90% and 3.65% against methanolic and water extract with 500gm plant sample.

Table 1: Successive value of Polygonum Species

S. No	Plant Name	Pt. ether Extract	Methanol Extract	Water Extract
1	*B. vaccinifolia*	1.253%	2.125%	2.527%
2	*P. polystachya*	3.629%	3.650%	4.210%
3	*B. macrophylla*	2.290%	3.015%	3.901%

Figure 1 Successive value of Polygonum Species

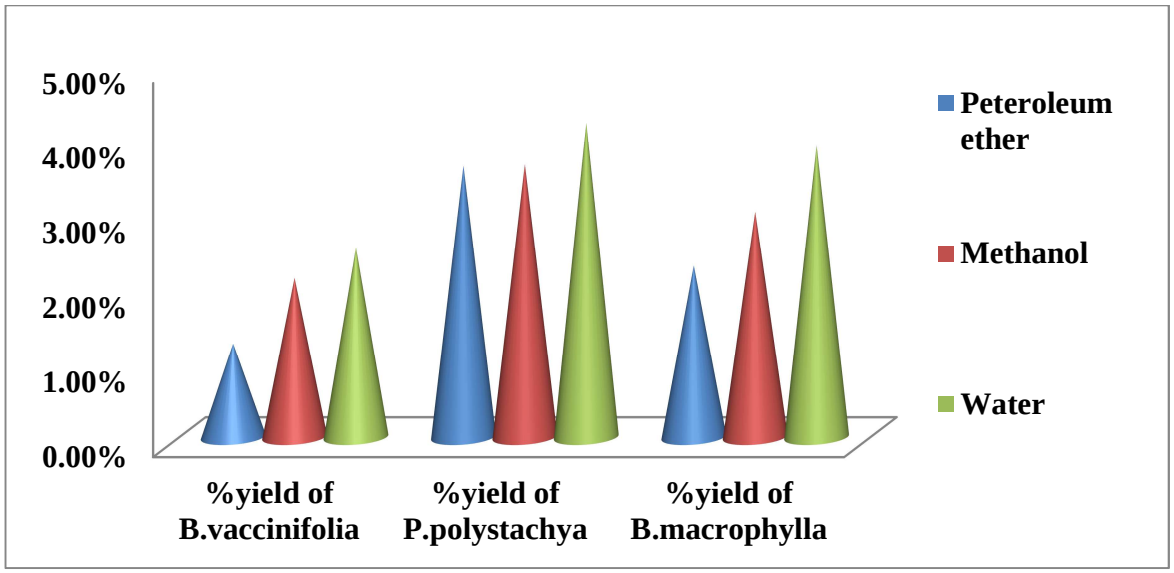


Figure 3.1 Thin layer chromatography qualitative analyses of three fractions of *B.vaccinifolia, P. polystachya*, and *B. macrophylla*

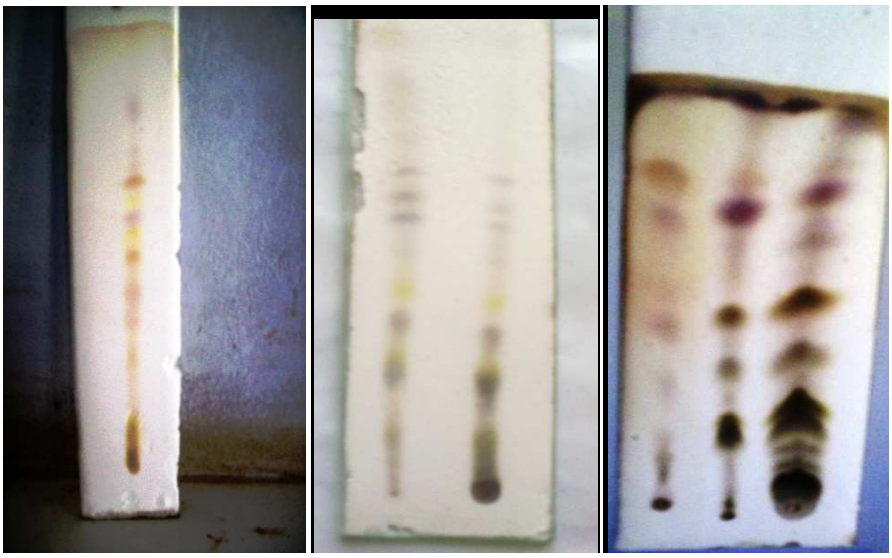


Table 2 Observations of thin layer chromatographic (TLC) studies of whole plants of *B.vaccinifolia, P. polystachya,* and *B. macrophylla,* (Benzene: Ethyl acetate) and (Toluene: Ethyl acetate)

S. No	Plants name	Extract	Mobile phase	No. of spot	Rf values	hRf. values
1.	***B. vaccinifolia***	Pt. ether	$C_6H_5CH_3$:$C_4H_8O_2$ (16:3)	3	0.24,0.62,,0.87	(24,62,87)
		Methanol	$C_6H_5CH_3$:$C_4H_8O_2$ (16:3)	5	0.09,0.25,0.62,0.81,0.87	(9,25,62,81,87)
		Water	$C_6H_5CH_3$:$C_4H_8O_2$ (16:3)	1	0.15	(15)
2.	***P. polystachya***	Pt. ether	C_6H_6:$C_4H_8O_2$ (16:2)	4	0.08,0.62,0.78,0.84	(8,62,78,84)
		Methanol	C_6H_6:$C_4H_8O_2$ (16:2)	5	0.09,0.37,0.62,0.78,0.90	(9,37,62,78,90)
		Water	C_6H_6:$C_4H_8O_2$ (16:2)	1	0.7	(7)
3.	***B. macrophylla***	Pt. ether	$C_6H_5CH_3$:$C_4H_8O_2$ (14:4)	4	0.29,0.33,0.65,0.75	(29,33,65,75)
		Methanol	$C_6H_5CH_3$:$C_4H_8O_2$ (14:4)	6	0.18,0.34,0.62, 0.68,0.78,0.82	(18,34,62,68, 78,82)
		Water	$C_6H_5CH_3$:$C_4H_8O_2$ (14:4)	1	0.47	47

Table 3: Phytochemical screening of different plants extracts

(P.E=Pet. ether extract, M.E= methanolic extract and W.E= water extract), (+)-Present, (-)-Absent

Plants		***B. vaccinifolia***			***P. polystachya***			***B. macrophylla***		
S. No	Test	P.E	M.E	W.E	P.E	M.E	W.E	P.E	M.E	W.E
1.	Carbohydrate/Sugar									
	(1)Molish's test	(-)	(+)	(+)	(-)	(+)	(+)	(-)	(+)	(+)
	(2)Fehling test	(-)	(+)	(+)	(-)	(+)	(+)	(-)	(+)	(+)
	(3)Benedicts test	(-)	(+)	(+)	(-)	(+)	(+)	(-)	(+)	(+)
2.	Glycoside's									
	Cardiac glycoside									
	(1)Keller Kiliani 's test	(-)	(+)	(-)	(-)	(+)	(+)	(-)	(+)	(+)
	(2)Legal s test	(-)	(+)	(-)	(-)	(+)	(+)	(-)	(+)	(+)
3.	Alkaloids									
	(1)Mayer's test	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	(2)Dragendroff's 's test	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
4.	Flavonides									
	(1)Alkaline reagent test	(-)	(+)	(+)	(-)	(+)	(-)	(-)	(+)	(+)
	(2)Lead acetate test	(-)	(+)	(+)	(-)	(+)	(-)	(-)	(+)	(+)
5.	Phenolic Compounds									
	(1)Ferric Chloride test	(-)	(+)	(-)	(-)	(+)	(-)	(+)	(+)	(+)
	(2)Nitric acid test	(-)	(+)	(-)	(-)	(+)	(-)	(+)	(+)	(+)
6.	Tannins									
	(1)Gelatin test	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(+)	(+)
	(2)Vanillin hydrochloride Test	(-)	(-)	(-)	(-)	(+)	(-)	(+)	(+)	(+)
7.	Saponin									
	(1)Froth test	(-)	(+)	(+)	(-)	(+)	(+)	(-)	(+)	(+)
	(2)Foam test	(-)	(+)	(+)	(-)	(+)	(+)	(-)	(+)	(+)
8.	Protein &Amino acid									
	(1)Xanthoproteic	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(+)
	(2)Ninhydrin	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(-)
9.	Phytosterol/Terpenoids									
	(1)Salkowski's test	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	(2)Liebermann Burchad 's test	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

Table 4 Quantitative analysis of total phenolic and total flavonoids

S. No	Plants	Extract	Phenolic content mg of *GA*/g of extract	Flavonoids content mg of *GA*//g of extract
1.	B. vaccinifolia	Methanolic	54.57	49.05
2.	*P.polystachya*	Methanolic	67.42	43.16
3.	*B.macrophylla*	Methanolic	76.14	51.55

Figure 1.1 Phenolic and flavonoids content present in different plants

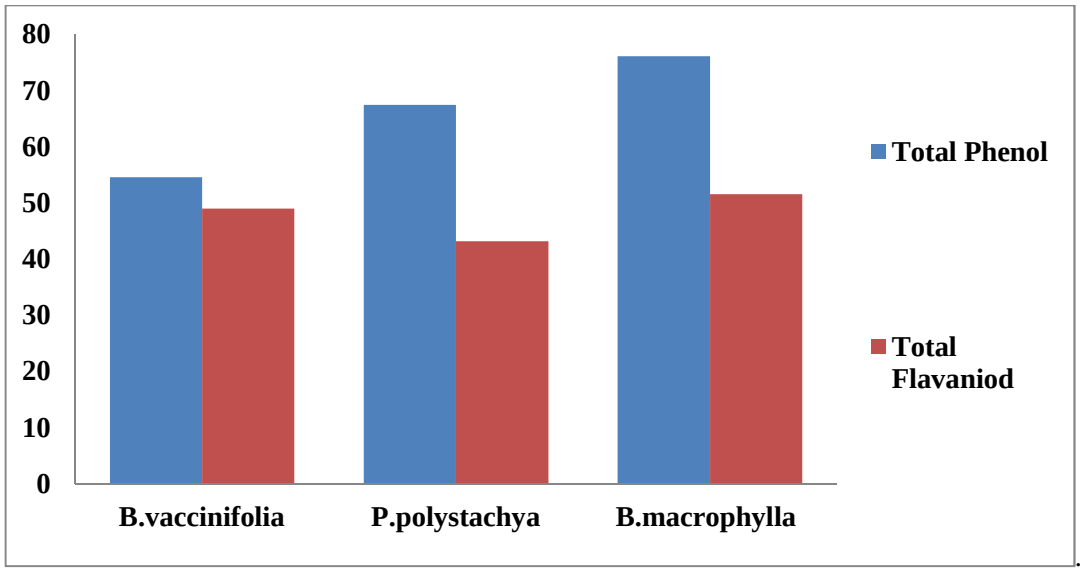


Fig. 1.2 Calibration curve of total phenolic content

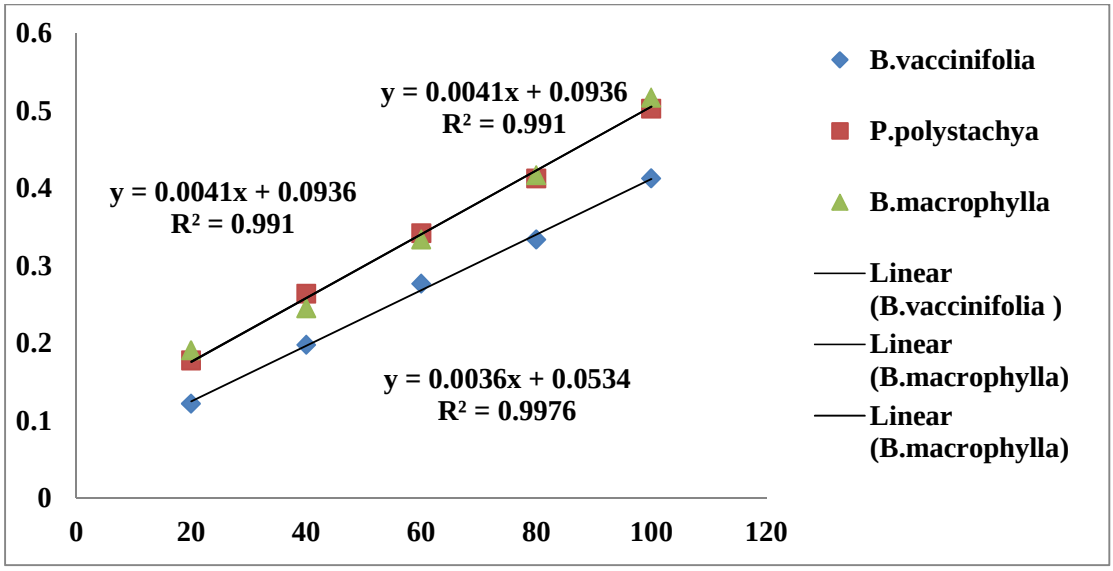


Table 5 Quantitative analysis of total tannins and total saponins

S. No	Species name	Tannins		Saponins
		Methanol extract	Water extract	Methanol extract
1.	*Persicaria polystachya*	13.15%	9.16%	35.8%
2.	*Bistorta macrophylla*	18.40%	16.79%	40.5%
3.	*B.vaccinifolia*	12.15%	9.50%	26.88%

Fig. 1.3 Calibration curve of total flavonoids content

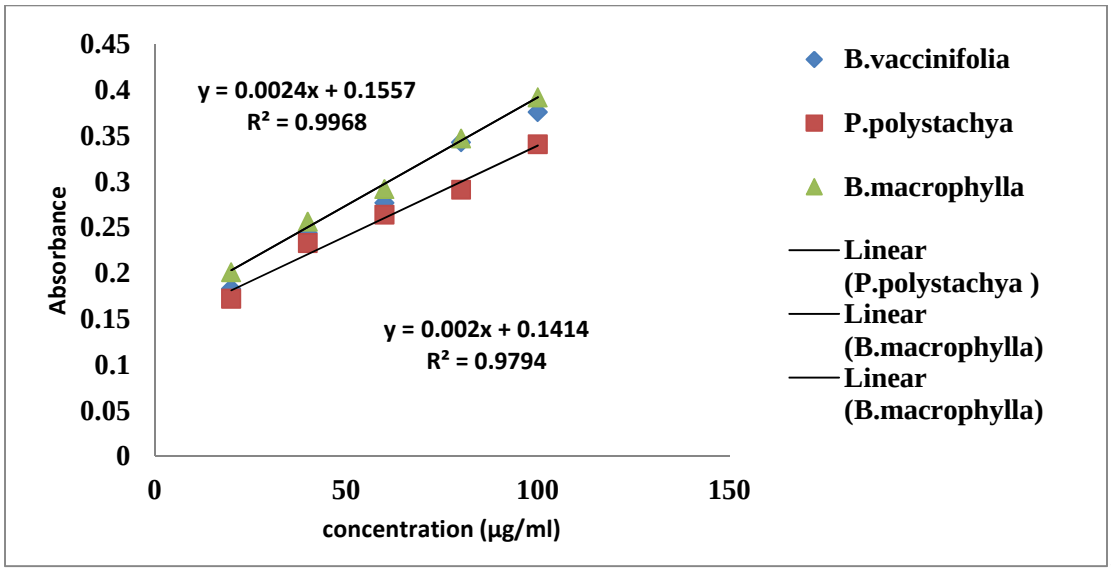
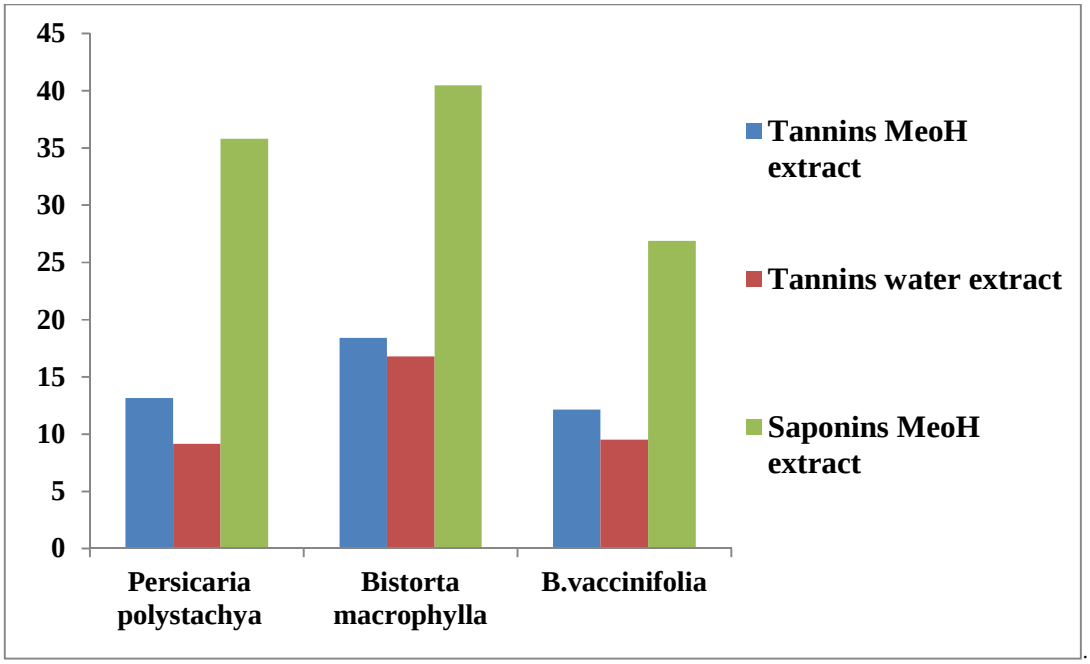


Figure 1.4 Tannins and saponins content present in different plant extract



Antioxidant activity (free radical scavenging activity):

The free radical scavenging activity of the methanolic extract of different Plant has been tested by DPPH radical method using Quercetin as a reference standard. The concentration ranged from 20–100 µg/ml. DPPH is very stable free radical. The antioxidant activity of Standard and different plant species in terms of inhibition (%).The highest Inhibition percentage of Quercetin standard is 85.12% in 100µg/ml concentration.

Table18: Absorbance and inhibition percentage at various concentration of In–vitro antioxidant activity of standard (*Quercetine*) and Polygonum Species

S. No	Concentration	*Quercetine*	*B. vaccinifolia*	*P. polystachya*	*B. macrophylla*
		Inhibition %	Inhibition %	Inhibition %	Inhibition %
1	20	36.94%	29.07%	34.93%	36.18%
2	40	49.52%	40.21%	44.14%	44.72%
3	60	56.62%	45.68%	52.78%	59.21%
4	80	71.97%	61.80%	63.33%	67.08%
5	100	85.12%	69.76%	80.90%	83.39%

Fig. 2: Absorbance and inhibition percentage at various concentration of In–vitro antioxidant activity of standard (*Quercetine*) and Polygonum Species

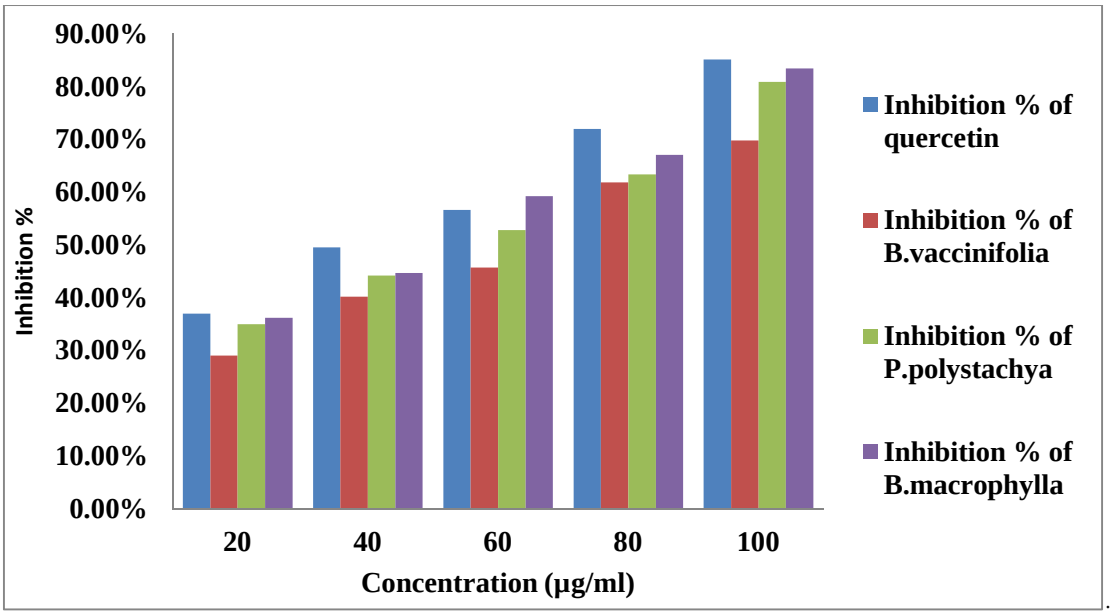


Fig. 2: Ic50 values (µg/ml) of Quercetine and different plants

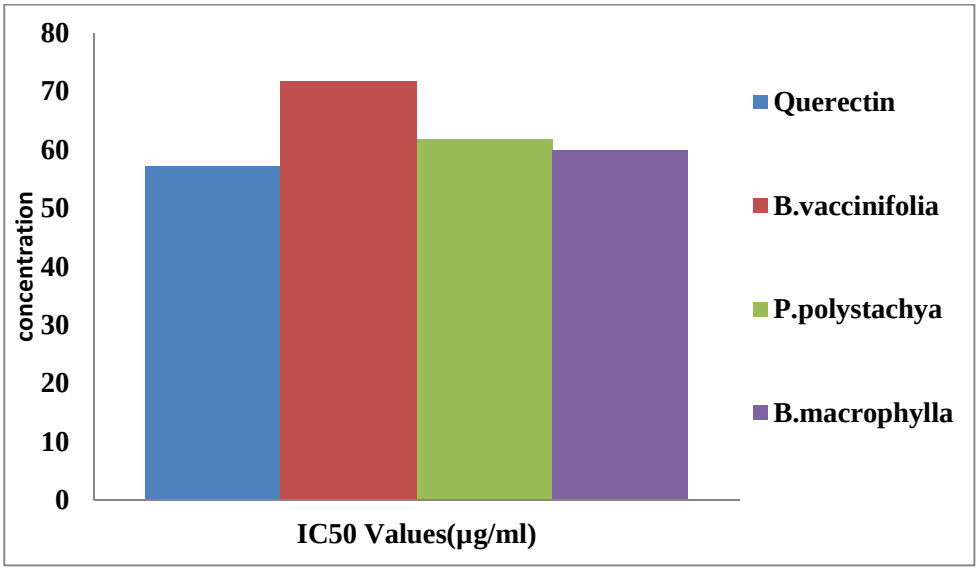


Table 5. Ic50 values (µg/ml) Quercetine and different plants

S. No	Sample name	IC_{50} value(µg/ml)
1	*Quercetin*	57.28
3	*B.vaccinifolia*	71.80
4	*P.polystachya*	61.80
5	*B.macrophylla*	59.95

CONCLUSION

It can be concluded that the different extract of the whole of *Bistorta macrophylla*, *B.vaccinifolia* and *Persicaria polystachya* possess potent antioxidant and phytochemical screening and rich sources of different medicinal and traditional uses. The present study was attempted for the first time to investigate the antioxidant and phytochemical activity of *B. macrophylla, B.vaccinifolia* and *P. polystachya* to search for newer, safer and more potent antioxidant agent and we herein delineate the results of our study. This analysis revealed that, the whole plant contained higher value of different secondary metabolites, which are used in different disease.

Acknowledgements
This work is financially supported by department of Pharmaceutical Chemistry, H. N. B. (A Central University) Srinagar Garhwal, Uttarakhand, India. The authors would like to thank Dr. R. M. Painuli, Taxonomist & Museum coordinator, deptt of Botany, HNBGU Srinagar, Uttarakhand, for plant identification.

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