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Ethnopharmacological Studies on Phytochemicals Obtained from *Skimmia laureola* (DC.) Zucc. ex Walp. of Pakistan.

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ABSTRACT

A detailed study regarding total antioxidant capacity (TAC), radical scavenging and antimicrobial effects of the essential oils from *Skimmia laureola* leaves (SL), stem (SS) and roots (SR) were investigated. Essential oils obtained by steam distillation process were subjected to various assays to determine antioxidant capacity. TPC values were found to be 9.07, 20.90 and 71.95 mg/L gallic acid equivalent and 4.08, 22.96 and 329 mg/L quercetin equivalent for SL, SR and SS, respectively. A significant correlation between the percent inhibition of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulpohonic acid) (ABTS) radical cation and the amount of essential oils viz R^2 = 0.9971, 0.9851 and 0.996 for SS, SL and SR respectively. The percent superoxide anion radical scavenging activity was found to be 51.75, 45.37and 56.5 percent for SL, SR and SS samples, respectively. The reducing power in terms of ferric reducing antioxidant power (FRAP) values were found to be 0.114, 14.33 and 1.2. mM $FeSO_4$ for SL, SR and SS respectively. All samples exhibited good metal chelating activities. The percent inhibition of the complex formation was found to be 80.37, 44.35 and 25.96 for SL, SR and SS respectively. The oils showed antimicrobial activities comparable to chloramphenicol. The data obtained from oils demonstrate the powerful antioxidative, radical scavenging and antimicrobial properties of the plant.

Keywords: Antioxidant Potential, Radical Scavenging, TAC, *Skimmia laureola*.

INTRODUCTION

Skimmia laureola is a shrub found throughout the temperate Himalayas from Murree to Mishmi and Khasia mountains. It is an extremely aromatic, gregarious, evergreen shrub. The smoke of burning leaves is supposed to purify the air (Nandkarni, 1982). Chromones and coumarins, including the new chromone, skimminin were isolated and characterized from *Skimmia laureola* (Waight *et al.,* 1987). Skimmia oil obtained from leaves is used in high grade perfume and as incense (Niad, 1997). Oil is antiseptic, effective against *Staphylococcus*, *Streptococcus* and leaves used to treat small pox (Skane, 2010), used in flavouring curries (Johanns, 2005). *Skimmia laureola* leaf paste mixed with cow's urine and the paste applied twice a day for 4 to 28 days for the treatment of psoriasis, leucoderma. Plant contains essential oil containing terpines, l-linalool, l-linalyl acetate, azuline and bergaptene. It also contains alkaloid skimnianin, furocoumarin, isopimpinellin, umbelliferone, laureoline (Ranaa *et al.,* 2010). Similarly the extracts of *Phyllanthus urinaria, Thevetia nerifolia, Jatropha gossypifolia Saraca asoca, Tamarindus indica, Aegle marmelos, Acacia nilotica, Chlorophytum borivilianum, Mangifera indica, Woodfordia fruticosa* and *Phyllanthus emblica* showed antimicrobial activity in a range of 75-1200 µg/ml.

The diverse antidisease activities claimed for *Skimmia laureola* extracts/oils and the increasing demand for antioxidant compounds from natural sources encouraged us to undertake a comprehensive study of the antioxidant and radical scavenging activities of the plant. The main objective of this study is to investigate and compare the total antioxidative capacity (TAC) and radical scavenging activities of extracts from root, leaves and stem of *Skimmia laureola*. TAC and radical scavenging activities were determined in terms of ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1996), ABTS, DPPH and superoxide anion radicals scavenging activities, total phenolic and total flavonoid contents and metal chelating activity.

MATERIALS AND METHODS

The plant material (leaves, roots and stem) of *S. laureola* was collected from Abbotabad, Pakistan. Water-cleaned and shade-dried plant material was subjected to steam distillation. The samples obtained were used either neat or in diluted

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form in various antioxidant and radical scavenging assays.

Antioxidant assays

Total soluble phenolic compounds were determined following the method of Singleton & Rossi (1965) using gallic acid as a standard phenolic. FRAP (Ferric reducing antioxidant power) values were determined following the method of Benzie & Strain (1996). Final results were expressed as FRAP values (mM $FeSO_4$. $7H_2O$). 2,2'-azinobis(3-ethylbenzothiazoline-6-sulpohonic acid) (ABTS) scavenging activity was determined by following the method of Re *et al.,*(1999) with minor changes. (2,2'-Diphenyl-1-picrylhydrazyl Radical Scavenging Activity) DPPH free radical scavenging activity of the plant oils was measured by using the method of Shimada *et al.,* (1992). Total antioxidant activity of the essential oils was determined according to the method employed by Mitsuda *et al.,* (1992). Superoxide anion radical scavenging activity was determined using the method of Nikishimi *et al.,* (1972). Metal (Ferrous ion) chelation by plant samples was estimated according to the method employed by Dinis *et al*., 1994.

RESULTS AND DISCUSSION

Percent yields and total phenolic content.

The percent yields and total phenolic contents of the sample oils were calculated and shown in Table 1. In comparison with SR and SL about 15 times greater percent recovery of oil was found for SR sample. SS oil of the plant was found richer in phenolic contents. It is quite obvious that in spite of low percent recoveries, the TPC values were quite good, showing richness of the oil samples with phenolic components. High TPC contents indicated high antioxidant and radical scavenging capacities for all the samples.

ABTS radical scavenging capacity and relationship between TEAC and TPC

ABTS radical cation produced as a result of reaction between ABTS and potassium persulfate in aqueous solution at physiological pH has considerable stability and sensitivity towards crude and specific antioxidants (Re *et al.,* 1999). The reduction potential of ABTS radical cation is very similar to that of hydroxyl radical cation. So in test environment it may be taken as equivalent to hydroxyl radical produced *in vivo* during certain disorders and metabolic reactions. ABTS radical scavenging ability of the test samples was evaluated using ABTS radical cation decolorization assay.

Table 1: Percent recovery, total phenolic content (TPC) of essential oils from leaves, roots and stem of *Skimmia laureola.*

Property	***Skimmia laureola***		
	Leaves(SL)	Roots(SR)	Stem(SS)
Weight of specimen (Kg)	2.200	1.765	1.560
Weight of oil (g)	0.50	0.50	1.50
Percent recovery	0.020	0.028	1.000
Color of oil	Clear yellowish	very light yellow	Clear light pale
TPC(mg/L GAE)	9.07	20.90	71.95

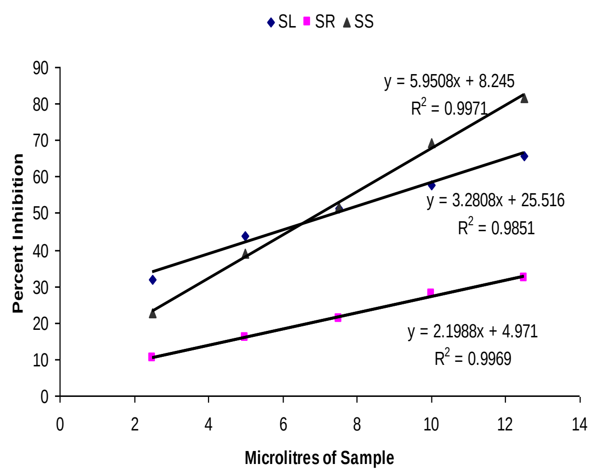

Fig., 1: Comparison of dose dependent percent inhibition of ABTS radical cation by essential oils from leaves, roots and stem of *S. laureola.*

Figure 1 shows the dose dependent percent inhibition of ABTS radical cation by the two samples. A significant correlation R^2 = 0.9971, 0.9851 and 0.996 for SS, SL and SR, respectively was found between percent inhibition and amount of the sample. Due to health-promoting effects of antioxidants a general recommendation to the consumer is to increase the intake of foods rich in antioxidant compounds e.g. polyphenols, flavonoids (Ahmad, 1995; Davies 2000; Dreosti 1991; Finkel 2000; Sies1982; Tiwari 2001; Lee *et al.,* 2003; Lee & Shibamoto (2000); Liu & Ng 2000 and Velioglue *et al.,* 1990). Phenolic compounds, especially flavonoids and anthocyanins, are very important antioxidants because of their natural origin and their ability to act as efficient free radical scavengers (Hertog, 1993; Hertog *et al.,* 1995 and Langley, 2000). Small berries have been reported to be rich sources of phenolic compounds such as phenolic acids as well as anthocyanins, proanthocyanidins, and other flavonoids, which display potential health promoting effects (Fukumoto & Mazza 2000; Hakkinen *et al.,*1999; Wang *et al.,*1996; Block *et al.,*1992; Bomser *et al.,* 1996; Feldman 2001 and Saito *et al.,* 1998).Total phenolic contents in terms of gallic acid equivalents of all the extracts were determined using Folin-Ciocalteu's method. The extracts showed high GAE values. The amount of total phenolics for SS, SL and SR samples were found to be 71.95, 9.07 and 20.90 mg/L gallic acid equivalent respectively. High values of TPC obtained for all the samples demonstrated presence of various phenolic acids and flavonoid components in these samples. It is also evident from the data that ABTS radical cation decolorization assay is more linearly related to TPC. Attempts have been made to derive a relationship between the phenolic contents and antioxidant activity. Controversial results have been obtained regarding a linear relationship between TPC and antioxidant activity (Faure *et al.,*1990).

The present study showed a relatively good relationship between TPC and antioxidant activity determined through ABTS radical cation decolorization assay and FRAP Assay. Non-acquisition of absolutely linear relationship between TPC and the two assays may be due to different response of different phenolics in Folin-Ciocalteau Reagent (Gazzani *et al.,* 1998) difference in the pH of the medium of assays and the reduction potential of the oxidized species. Furthermore the antioxidant activity strongly depends upon the chemical structure of phenolic compounds. Therefore no definite quantitative relationship could be obtained for general application to all the plant extracts.

DPPH, Lipid Peroxyl and Superoxide Anion Radicals Scavenging Activities

DPPH and lipid peroxide free radicals have been used to evaluate reducing properties and to assess free radicals chain breaking abilities of phyto-chemicals. Figure 2 demonstrates the kinetics of DPPH radicals scavenging by SS, SL and SR. All EOs showed time dependant quenching of DPPH radicals. SL sample was found to be a better quencher of DPPH radicals than other EOs. The absorbance continued to decrease with almost a uniform gradient throughout the time span of 30 minutes showing the presence of a good amount of slow reacting antioxidant components in both the mixtures.

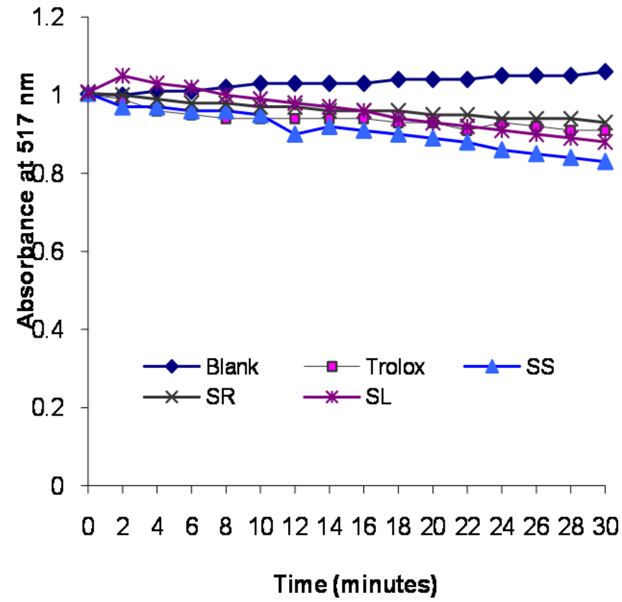

Fig., 2: Kinetics of DPPH radical scavenging by SL, SR and SS essential oils

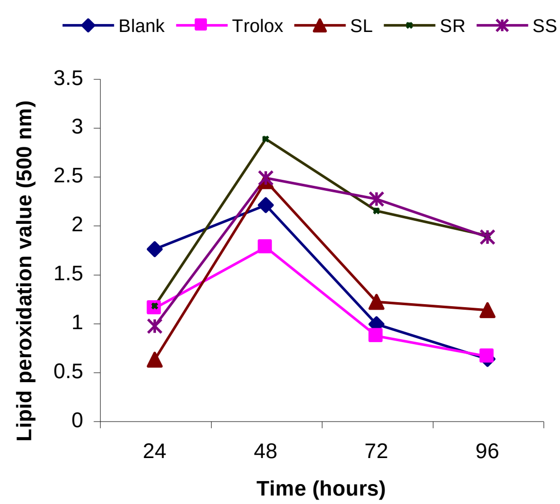

Fig., 3: lipid per oxidation by SL, SR and SS essential oils

In biosystems, unsaturated fatty acids are always at stake due to free radicals attack on the bio-membranes which results in membrane lipid per-oxidation, decrease in membrane fluidity, loss of enzymes and receptor activity and damage to membrane proteins leading to cell inactivation (Heinonen *et al.,* 1998). Many disorders like hyper-glycaemia have been ascribed to development of oxidative stress due to increased lipid peroxide production (Dean and Davies, 1993). Lipid peroxidation values of the extracts were found using linoleic acid emulsion system. Linoleic acid after its aerial oxidation to peroxyl radicals converts ferrous to ferric form. The extent of conversion is assessed through Iron (III) complex with thiocyanate, spectrophotometrically. The antioxidative components in proportionate to their amount halt this conversion by trapping peroxyl radicals. Figure 3 shows that all samples had considerable resistance to lipid per oxidation which is quite comparable with that of trolox (10 µM).

Superoxide (SO) anion radical is one of the important ROS which is produced first after oxygen is taken inside the body. The subsequent dismutation of SO leads to the formation of other injurious ROS. So the capacity of samples to scavenge ROS can play a very crucial role in determining the overall strength of antioxidant activity. The percent superoxide anion radical scavenging activity was found to be 51.75, 45.37and 56.5 percent for SL, SR and SS sample, respectively.

Reducing and Metal Chelating Activities

FRAP assay was employed to estimate the ferric reducing activity of the samples using $FeSO_4$ as the standard reducing agent. At low pH, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to intense blue colored ferrous form can be examined by measuring the change in absorption at 593nm. Being a non specific reaction any half reaction that has lower redox potential, under the test conditions, than that of Fe(III)/Fe(II) reaction will convert Fe(III) to Fe(II). The change in absorbance reflects cumulative reducing power of the electron donating antioxidants present in the reaction mixture. The FRAP values were found to be 0.114, 14.33 and 1.2. mM $FeSO_4$ for SL, SR and SS respectively. The metal chelating activity was found by determining the chelating activity of the sample with ferrous ion in the presence of ferrozine (a ferrous chelating agent). In the presence of chelating components of the sample the formation of Fe(II)-ferrozine complex, which may be monitored at 562 nm spectrophotometrically is halted. The percent inhibition of the complex formation was found to be 80.37, 44.35and 25.96 for SL, SR and SS respectively.

Antibacterial and antifungal activity

Agar well diffusion method was performed to calculate zones of inhibition for the samples. The results obtained (Table-2) indicated that all the samples showed varied affectivity against all the organisms tested except *Salmonella typhimurium* and *Rhodutrula minuta*

Table 2: Antibacterial and antifungal activity of essential oils from leaves (SL), roots (SR) and stem (SS) of *S. laureola*

	Zone of Inhibition (cm)			
Strains / Treatments	**SL***	**SR***	**SS***	**Chloramphenicol** (1µg/µL)
Bacillus cereus	1.2	1.9	2.2	1.0
Staphylococcus aureus	1.2	2.0	2.1	1.2
Salmonella typhimurium	1.4	1.8	1.8	2.4
Escherichia coli	2.4	2.0	1.9	0.8
Pseudomonas aeruginosa	0.8	2.1	1.6	2.0
Enterobacter aerogenes	1.2	1.2	2.0	1.6
Streptocococus equi	1.1	1.4	1.8	1.8
Micrococcus roseus	2.0	1.2	2.0	2.0
Aspergillus niger	2.2	2.1	3.0	2.5
Penicillium chrysogenum	2.6	2.1	1.8	2.0
Saccharomyces cerevisiae	2.0	2.2	2.2	2.1
Rhizopus oligosporus	1.8	2.2	1.6	2.0
Rhodutrula minuta	2.0	1.6	2.2	2.6

*100 microlitres of each oil and the standard was used in the experiment

CONCLUSION

The data presented here shows that *S. laureola* extracts have great antioxidant and radical scavenging activity and thus may be used as a good source of natural antioxidants. The *in vivo* efficacy of *S. laureola* oils against diabetes mellitus or other degenerative diseases may be partially attributed to radical scavenging and antioxidant activity of the plant. As the oils also showed significant activity against tested organisms, they could be potential sources of new antimicrobial agents.

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