



**Oxidation Management Potential of Essential Oils of *Mentha longifolia*,  
*Angilica glauca*, *Artemisia meritima*, *Cerdu deodara*, *Thymus serypelleum* and  
*Sassura* from Higher Altitude of Azad Jammu and Kashmir**

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**ABSTRACT**

Medicinal plants have long enjoyed their reputation as healing agents. In the current study lappa *Mentha longifolia*, *Angilica glauca*, *Artemisia meritima*, *Cerdu deodara*, *Thymus serypelleum* and *Sassura* was selected medicinal plants for extraction of essential oils by hydrodistillation method. The ability of scavenging free radicals of the oil extracts of above medicinal plants was determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH), reducing power, as well as total phenolic contents (TPC) and total flavonoid contents (TFC) was also determined. The results showed that the oil extract of *Artemisia meritimia*, *Thymus serypellum* and *Mentha longifolia* had significant antioxidant activity. Thus, the study suggests that *Artemisia meritimia*, *Thymus serypellum* and *Mentha longifolia* has a better source of natural antioxidants, which might be helpful in preventing the progress of oxidative stress.

**Keywords:** *Mentha longifolia*; *Angilica glauca*; *Artemisia meritima*; *Cerdu deodara*; *Thymus serypelleum*; *Sassura lappa*; Essential oils; Antioxidant assays

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**INTRODUCTION**

Medicinal plants are not only provide opportunity for health care but also important for millions of people for purpose of traditional medicine. On the basis of these results, it is suggested that the medicinal plants are rich source for antimicrobial peptides/proteins and in future, may be used for industrial scale extraction and isolation of antimicrobial compounds particularly peptide/protein which may find place in medicinal industry as constituent of antibiotics.

Traditionally, essential oils from many medicinal plants have been used for treatment of many diseases all over the world. Essential oils from the medicinal plants have growing market value due to its wide range of applications. Theses essential oils have been used in beverages and food industry and in cosmetics due to its fragrances. Such oils cover a broad spectrum of biological activity which has led to an increased interest among researchers. In recent years extensive research is there to explore the antimicrobial activity of essential oils. Some essential oils have displayed antimicrobial activity and some have to be more effective than others (Afzal *et al.*, 2014).

It is unclear to pinpoint exactly when man first discovered the medicinal virtues of herbs and plants (Barreto *et al.*, 2006). *Angilica gluca* is native herbious plant that found in North West areas of Pakistan. *Artemisia maritima* is also known as sea worm wood, which is found in coastal areas. Herbal medicine remains the most important field of medicine in many rural parts of the world for the treatments of various ailments. It is essential to determine whether the potential medicinal ability of these plants match their folklore reputation before they can be promoted as herbal medicine (Ali *et al.*, 2001; El-seedi *et al.*, 2002; Sharma, 2010).

*Thymus serypellum* is belongs to Family *Lamiaceae* (angiosperm). It is a short in length, woody plant that has creeping stems with tiny and shiny leaves. It is shortest among growing grasses and other aromatic herbs that have tiny pink flowers. *Mentha longifolia* (mint) belongs to Family *Lamiaceae*.

*Cedrus deodara*, as found in northern areas so known as Himalayan cedar. Its height is 50 to 70 feet. When young it is pyramidal with a broad base. Its growth rate is very fast around 3 feet per year. Its leaves are foliage in nature that is silvery blue-green.

Particularly essential oils having long chain fatty acids with C<sub>20</sub> and C<sub>22</sub> have shown hepato- protective agent in aging mammals and in animals. The reason behind is that antioxidants are important to human physical well-being. As oxygen is a potentially toxic element that can be transformed by metabolic activity into more reactive forms such as singlet oxygen, super oxide, hydrogen peroxide and hydroxyl radicals, collectively known as reactive oxygen species (Chill, 2006). *Saussurea lappa* belongs to Family - *Asteraceae*. As a component of traditional Tibetan medicine, it is used as anti-inflammatory drug. It is investigated that it inhibits the mRNA expression and reducing nitric oxide production.

In the recent year many multiple resistance drugs has been developed due to extensive use in the treatment of infectious diseases. As the result antibiotics are associated with advance effects on host-like hypersensitivity. So there is a need to develop alternative antimicrobial drugs for the treatment of many diseases from other sources, such plants. Natural products from higher plants may be a new source of antimicrobial agents' that have novel mechanism of action (Maria *et al.*, 2007).

## MATERIAL AND METHOD

The essential oils from the leaves and stem of selected medicinal plants were extracted by hydro distillation method and different plant name presented there.

- I *Angilica gluca*
- II *Cedrus deodara*
- III *Mentha longifolia*
- IV *Skima laureda*
- V *Artemisia meritimia*
- VI *Sassurea lappa*
- VII *Thymus serypellum*

### Extraction of oils

Different types of analytical methods are used for the extraction the volatile compounds from medicinal plant material. Solvent extraction, steam distillation and hydro-distillation methods were used for extraction of essential oils from the given medicinal plants. In addition to the standard methods for extraction of essential oils from the plants, the technique of hydro distillation was found acceptance because it is simple and inexpensive. (Afzal *et al.*, 2014)

### Hydrodistillation method

Take (100g) fresh aerial parts of medicinal plants and ground them, then subjected hydrodistillation for 3 h, using a Clevenger-type apparatus as recommended by British Pharmacopeia (1988.). Briefly, the aerial parts of plant were immersed in water and boiled, after which the essential oil was evaporated together with water vapors and finally collected in a condenser (Fakhar *et al.*, 2005).

### Antioxidant activity of plant extracts

Antioxidant activity of plant extracts were measured by different antioxidant assays including;

#### DPPH radical scavenging assay

DPPH assay was done by using 1-diphenyl-2-picrylhydrazyl reagent and BHT was used as standard.

$$I \% = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$$

Extract concentration providing 50 % inhibition (IC<sub>50</sub>) was calculated from the graph plotted inhibition percentage against extract concentration (Bozin *et al.*, 2006).

#### Determination of reducing power

Reducing power of the given plant sample was determined by using sodium phosphate buffer of pH 6.6 and potassium ferricyanide [K<sub>3</sub>Fe (CN)<sub>6</sub>]. Absorbance was taken at 700 nm. Ascorbic acid was as standard. (Tandrima *et al.*, 2013). Increase in reducing power (%) = A of sample - A of blank / A of blank × 100

### Antioxidant activity determination by linoleic acid oxidation system

The antioxidant activity of sample extract was determined by measuring % age inhibition of peroxidation by using the linoleic acid system that was reported by Iqbal and Bhanger (2005). Take 5mg of sample extract and added to a solution mixture of linoleic acid (0.13 mL), 98 % ethanol (10mL) and 10 mL of 0.2 M sodium phosphate buffer (pH 7.0),and dilute it by adding 25mL distilled water. The solution was incubated at 40 °C and the degree of oxidation was measured by following thiocyanate method (Yen *et al.*,2000) with 10 mL of ethanol (75 %), 0.2 mL of an aqueous solution of ammonium thiocyanate (30 %), 0.2 mL of sample solution and 0.2 mL of ferrous chloride (FeCl<sub>2</sub>) solution (20 mM in 3.5 % HCl) being added sequentially. After 3 min of stirring, take the absorption at 500 nm. A control was performed with linoleic acid but without extracts.

### Synthetic antioxidants

Butylated hydroxytoluene (BHT) and ascorbic acid (200 µg/mL) was used as positive control. The maximum peroxidation level measured as 175 hours (7 days) in the sample those contain no antioxidant component was used as a test point.

### Percent inhibition

% inhibition of synthetic antioxidant=  $100 - (\text{Abs. increase of sample at 175h} / \text{Abs increase of control at 175h}) \times 100$

### Statistical analysis

The means  $\pm$  SD of antioxidant activities of samples of essential oils from the medicinal plants were calculated. The average representation was assisted with graphical representation (Steel *et al.*, 1997).

## RESULTS AND DISCUSSION

### Determination of reducing power

Essential oils have reducing potential and good antioxidant activities. In reducing power assay ferric ions are reduced to ferrous ions and with it change in color from yellow to bluish green. The intensity of color depends on the reducing potential of the compounds present in medium. Greater the intensity of the color, greater will be the absorption; consequently, greater will be the antioxidant activity (Zou, Lu and Wei, 2004). The reducing power is a good index of antioxidant compounds in any sample. A quite good profile of reducing power was recorded in *Sassurea lappa* and *Artemisia merutummuua* while minimum reducing power %age was recorded of *Cedrus deodada*. Reducing power content was studied by using the method as described by Yen *et al.*, (2000). Highest reducing power was observed in *Sassurea lappa* that was comparable with other oil extracts.

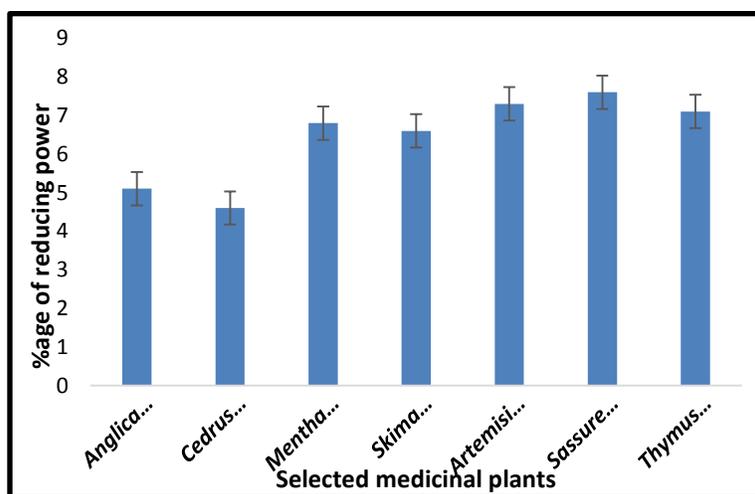


Figure 3: % of reducing power of essential oils

### DPPH activity

DPPH is very stable organic free radical with deep violet color which gives absorption within 515-528 nm. It loses its purple color and became yellow. As the concentration of phenolic compounds or the degree of hydroxylation of

the phenolic compounds increases DPPH radical scavenging activity increases (Sanchez-Moreno, Larrauri and Saura-Calixto, 1999).

Because this radical is very sensitive to active ingredients. So large number of samples in a very short time can accommodate. This assay is used for measuring radical scavenging activity of different plant extracts. However due to specificity of radical applied, which are not oxygen related radical species, antioxidant activity assessed can be related to them only. Essential oils and extracts showed excellent radical scavenging activity with  $IC_{50}$ .

The free radical scavenging activity of ethanol extracts was superior to that of essential oil. Furthermore, 80% ethanol and methanol extracts exhibited more scavenging activity than absolute ethanol extracts (Mata *et al.*, 2007).

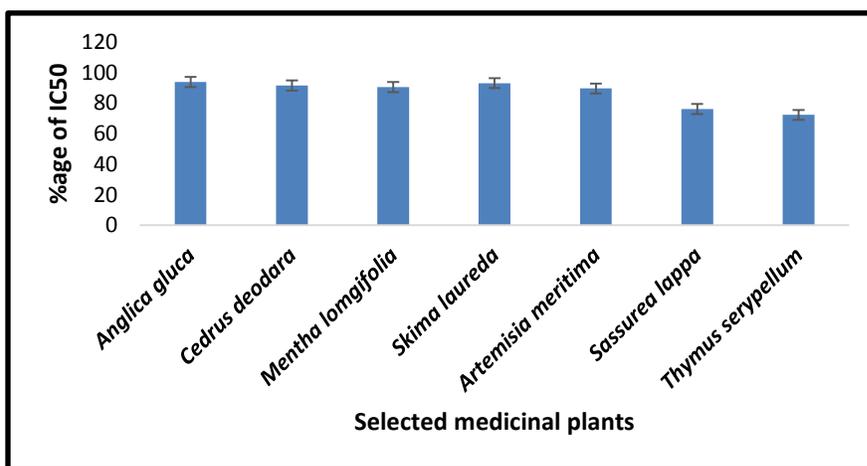


Figure 4:  $IC_{50}$  of essential oils

Free radical scavenging activity of *Sassurea lappa* (old) is maximum while free radical scavenging activity of *Mentha longifolia* is minimum. Similarly *Sassurea lappa* has maximum percentage of inhibition and *Mentha longifolia* has minimum percentage of inhibition.

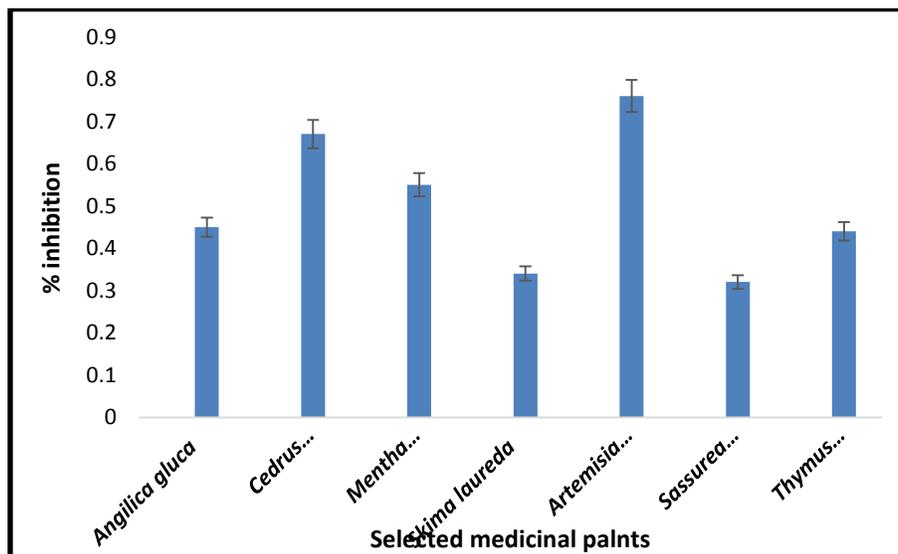


Figure 5: % inhibition of essential oils

### Antioxidant activity determination of linoleic acid system

The antioxidant activity has also been assessed as ability to prevent from oxidation. Therefore, inhibition of linoleic acid oxidation was also used to assess the antioxidant activity of oil extract of medicinal plants. The antioxidant activity of different oil extract was determined by inhibition of peroxidation in linoleic acid system using thiocyanate method (yen *et al.*, 2000). Linoleic acid is polysaturated fatty acid, which oxidizes  $Fe^{2+}$  to  $Fe^{3+}$ .  $Fe^{3+}$  form make a complex with SCN<sup>-</sup>. The absorbance was measured at 500 nm. Higher the absorbance higher will be the concentration of peroxides formed during the reaction, which lower the antioxidant activity.

All the oil extract exhibited appreciable inhibition of peroxidation ranging from 8% to 4%, and were compared with BHT (Butylated hydroxyl toluene, standard). *Artemisia meritimia* exhibited high peroxidation inhibition while *Skima laureda* and *Thymus serypellum cerypellum* have minimum peroxidation inhibition.

### CONCLUSION

It is concluded from this research work, the essential oils of different medicinal plants have significant antioxidant activity against different pathogens, and have a potential to develop immune system against different disease causing pathogens. These oils have such chemical compounds that help to develop immunity against different germs. The extraction of essential oil and separation of bioactive components from indigenous resources and their utilization as potential natural food preservatives have economic value. However, further investigation require more information in *in vivo* and *in vitro* studies to establish which components of essential oil have valuable information on the composition and antioxidant attributes of essential oil from Pakistan.

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