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Full Length Research Paper

Phytochemical, antioxidant and cytotoxic activities of *Periploca aphyla* and *Mentha longifolia,* selected medicinal plants of District Bannu, Pakistan

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***Mentha longifolia* and *Periphloca aphyla* are a well known species widely applied in medicine as a constituent of various drugs, and are very liked in aromatherapy. The current study was desighned to investigate the phytochemical screening, cytotoxic and antioxidant capacity of methanolic extract of *Mentha longifolia* and *Periphloca aphyla*. The methanolic extract of *Mentha longifolia* via qualitative analysis revealed the presence of terpenoids, saponins and flavonoids while anthraquinones, coumarins, cardiac glycosides, tannins, and phlobtannins remained absent. The extract also showed maximum free radical scavenging and cytotoxic potential in various concentrations in *Mentha longifolia* and *Periphloca aphyla*. The results revealed that methanolic extract of these plants contain a remarkable antioxidant and cytotoxic activity due the presence of bioactive constituents.**

Key words: Antioxidant, cytotoxic, *Mentha longifolia,* phytochemical screening.

INTRODUCTION

Today there has been a keen interest in the therapeutic activities of medicinal plants as antioxidants in lowering such free radical induced tissue injury. Besides well reported and traditionally practiced natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (for example, rosemary and sage) are already exploited commercially either as antioxidant additives or a nutritional food supplements (Schuler, 1990). Similarly, many other plant categories have been reported in the search for novel antioxidants (Chu, 2000; Mantle et al., 2000; Koleva et al., 2002; Oke and Hamburger, 2000) but generally there is still a need to find more information relating to the antioxidant activity of plant species.

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It has been studied that the antioxidant property of plants might be due to their phenolic substances (Cook and Samman, 1996). Flavonoids are a class of polyphenolic substances with reported properties including free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel, 1995). Some reports suggest that the biological actions of these substances are associated with their antioxidant potential (Koleva et al., 2002). An easy, fast and sensitive plan for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically.

In the presence of an antioxidant, DPPH radical receives one more electron and the absorbance declines (Koleva et al., 2002). In particular, despite extensive application of wild plants as medicines in Iran, the literature consist few evidences of antioxidant activity and chemical composition of these plants. In living systems, free radicals are emerged as part of the body's normal

metabolic system, and the free radical chain reactions are commonly produced in the mitochondrial respiratory chain, liver mixed function oxidases, through xanthine oxidase activity, atmospheric pollutants and from transitional metal catalysts, drugs and xenobiotics. Moreover, chemical mobilization of fat stores under different circumstances like lactation, exercise, fever, infection and even fasting, can lead to enhanced radical activity and damage.

Free radicals or oxidative damage now exhibits the fundamental mechanism underlying a number of human neurologic and other abnormalities. Oxygen free radical can generate peroxidation of lipids, which in turn activates glycation of protein, inactivation of enzymes and modification in the structure and function of collagen basement and other membranes, and have a role in the long-term complication of diabetes. The beneficial medicinal effects of plant materials are typically caused from the combinations of secondary metabolites found in the plant. The medicinal activities of plants is distinctive to particular plant classes or groups and are components with this concept, as the combination of secondary metabolites in a particular plant is taxonomically distinct. Antioxidant based dugs/ formulations for the prevention and curing of complicated diseases such as atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer have emerged during the last 3 decades (Mantle et al., 2000; Koleva et al., 2002). This has caught a great deal of research interest in natural antioxidants. Subsequently, a worldwide trend towards the application of natural phytochemicals found in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables have enhanced. The present study was arranged to (i) phytochemical inversion of both extracts, (ii) cytotoxic activity and, (iii) antioxidant potential of *P. aphyla* and *M. lonifolia*.

MATERIALS AND METHODS

Plant collection

Plant of *P. aphylla* and *M. longifolia* were collected from the main Township campus of University of Science and Technology, in Bannu, Khyber Pakhtunkhwa, Pakistan in the month of July, 2010. The plant was identified by Taxonomist Prof: Abdur Rehman, Chairman Department of Botany, Government Post Graduate College Bannu. The plant materials were washed with deionised water and were shade dried at room temperature for two weeks, chopped and grinded mechanically with mesh size 1 mm.

Preparation of plant extract

A 2 kg powder of each *P. aphylla* and *M. longifolia* was extracted in 3 l of 70% methanol by random shaking. After a week, the extract was filtered by using Whitman filter paper No. 1. After filtration, the filtrate was further concentrated by using rotary vacuum evaporator at 38°C in order to get the methanolic crude extract of the plant. The methanolic crude extract was stored at 4°C in the refrigerator for further phytochemical studies *in vitro* investigation.

Phytochemical studies

Presence of various chemicals in each fraction was carried out by using standard procedures. Qualitative studies for flavonoids, alkaloids, terpenoids and saponins were carried out according to Harborne (1973). Tannins were determined as described by Pan et al. (2008). In addition, coumarins, cardiac glycosides, anthraquinones and phlobatanins were performed according to the method described by Trease and Evans (1972).

DPPH radical scavenging activity

The DPPH assay was performed according to the standard procedure of Gyamfi et al. (1999) with little modifications. The fresh stock solution was prepared by dissolving 3 mg DPPH with 100 ml of methanol and then stored at 20°C. The working solution was obtained by diluting DPPH solution with methanol to obtain an absorbance of about 0.980 (±0.02) at 517 nm using the spectrophotometer. A 900 µl aliquot of this solution was mixed with 100 µl of the plant extract at varying concentrations (50, 100, 150, 200, 250 µg/ml in respective solvent). The solution in the test tubes were shaken well and incubated in the dark for 30 min at room temperature. Then the absorbance was taken at 517 nm. The EC_{50} of scavenging activity was calculated from the percentage of DPPH radical scavenged with the equation:

Scavenging effect (%) = [(control absorbance-sample absorbance) / (control absorbance)] × 100.

IC_{50} value is the effective concentration that could scavenge 50% of the DPPH radicals. Ascorbic acid standard was applied as positive reference. Each extract was assayed in duplicate.

Cytotoxic brine shrimp bioassay

Cytotoxic activities of methanolic crude extract of *M. longifolia* (Linn) and *P. aphyla* was carried out according to the standard procedure of (Meyer-Albert et al., 1992). Sample was prepared by dissolving 5 mg of crude plant extract in respective solvent (methanol) to form stock solution of 5 mg/1 ml in methanol and further diluted into 500 µg/ml, 1000 µg/ml and 1500 µg/ml. 28 g sea commercial sea salt (sigma) was dissolved in one liter of dH_2O with constant stirring for 2 h. Brine shrimps were hatched in two compartment rectangular tray having sea salt saline. One side of the compartment was made lightened. Eggs were sprinkled in dark compartment of tray and after 24 h of shrimps hatching larvae was collected by pipette from the lightened side. 0.5 ml of each solution (500, 1000 and 1500 µg/ml) was put in vials and evaporated the solvents. Residues was redissolved in saline of 2 ml. 8 shrimps were transferred to each vial and increased the volume up to 5 ml and incubate at 25 to 28°C. After 24 h of incubation, survivors were counted with help of 3× magnifying glass and calculation was done using Abbot's formula.

% Death = (Sample – control / control) × 100

LD_{50} was determined through prism graph pad software.

RESULTS AND DISCUSSION

Phytochemical analysis of *Mentha longifolia* (Linn.) and *Periphloca aphyla*

Phytochemical screening provides basic information about

Table 1. Phytochemical composition.

Samples	Flavonoids	Alkaloids	Terpenoids	Coumarins	Saponins	Tannins	Cardiac glycosides	Anthraquinone	Phlobatannins	Steroids	Phenolic compounds
PAME	+	+	+	-	+	-	+	-	-	+	+
MLME	+	+	+	-	+		-	-	-	-	-

+, -, presence and absence, respectively.

the medicinal importance of the plant extracts. Secondary metabolites including flavonoids, terpenoids, alkaloids, coumarins, saponins, tannins, anthraquinones, cardiac glycosides and phlobatannins possess antioxidant, anticancer and anti-inflammatory activity. Tannin possessed spasmolytic activity in smooth muscles cells, free radical scavenger and antioxidant. Flavonoids have antioxidant and antimicrobial properties while saponins are glycosides possessed antimicrobial and inhibit Na^+ efflux, by blockage of the entrance of the Na^+ out of the cell, reducing congestive heart failure (Gryglewski et al., 1987), alkaloids have possessed analgesic, antisplasmodic and bactericidal activities, antioxidant and are useful in renal disorder (Okwu, 2001; Abou-Donia et al., 2008). The current investigation showed the presence of certain constituents in methanolic extract of *M. longifolia* (Linn.). The *Mentha longifolia* (Linn.) (MLME) revealed the presence of flavonoids, terpenoids, alkaloids and saponins, while tannins, phlobatannins, cardiac glycosides, coumarins and anthraquinones were found absent while methanolic extract of *P. aphylla* (PAME) consisted of alkaloids, flavonoids, saponins, terpenoids, steroids, phenolic compounds and cardiac glycosides; whereas tannins, coumarins, anthraquinones and phlobatannins were found absent (Table 1). Similar phytochemicals were reported during characterization of medicinal plants (Pan et al., 2008). Similar results were reported by various studies that methanolic fraction possesses highest amount of phenolic and poly phenolic compounds (Sofowara, 1983; Mustafa et al., 2010)

Antioxidant activity

Oxidation is a necessary process of living things for energy production; however during normal metabolism, oxygen consumption, through many enzymatic systems, produces reactive free radicals (RFR). In small amounts, these ROS are beneficial in signal transduction and growth regulation. However, large amount of ROS produced oxidative stress, attack many molecules such as protein, DNA and lipids (Halliwell, 1999). DPPH free radical scavenging procedure has been widely in practice for studying antioxidant potential.

Figure 1 shows the % scavenging activity of *P. aphylla* methanolic extract (PAME) for free radicals of DPPH. Significant scavenging activity was observed by various concentration of *P. aphylla* methanolic extract with increasing concentration (50 < 100 < 150 < 200 < 250 µg/ml). Similar result was presented by various concentration of ascorbic acid used as a reference compound (50 < 100 < 150 < 200 < 250 µg/ml). Similarly, a remarkable scavenging activity was observed by various concentration of *M. longifolia* methanolic extract with increasing concentration (50 < 100 < 150 < 200 <250 µg/ml). Similar result was presented by various concentration of ascorbic acid used as a reference compound (50 < 100 < 150 < 200 < 250 µg/ml) as shown in Figure 2.

Data of the present study revealed that *P. aphylla* methanolic extracts show marked scavenging potential. Our result shows similarity with the investigations of Hagerman et al. (1998) and Falleh et al. (2008), who reported that medicinal plants markedly scavenge free radicals. The antioxidant potential of various fractions of both plants could be due to the presence of plant bioactive phenolic and polyphenolic compounds which significantly reduce the free radicals which cause oxidative stress. The results obtained by Duenas et al. (2006) and Kilani et al. (2008) also support our findings. The data of this work suggests that the methanolic extract of *M. longifolia* (Linn.) and *P. aphylla* has capacity to act as antioxidant agent.

Cytotoxic screening

Preliminary screening of plant extract through cytotoxicity provides helpful/positive information about the antitumor activity of the extract for the future use. Cytotoxic effect of (PAME) and MLME

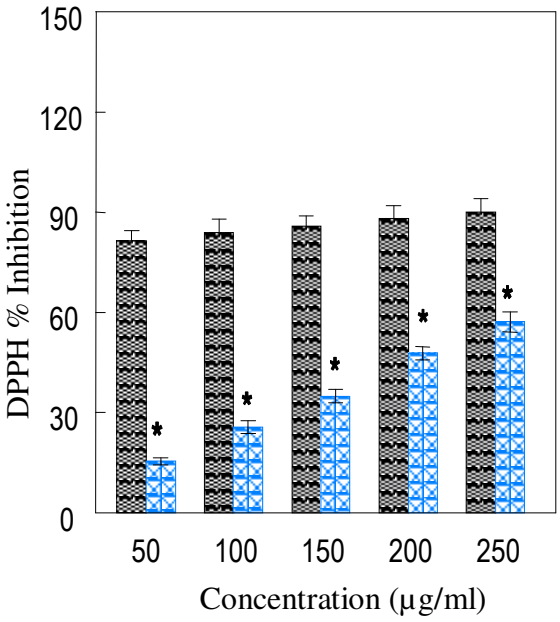

Figure 1. DPPH free radical scavenging of *Periploca aphylla* (Grey color) methanolic extract (PAME) and ascorbic acid (blue color).

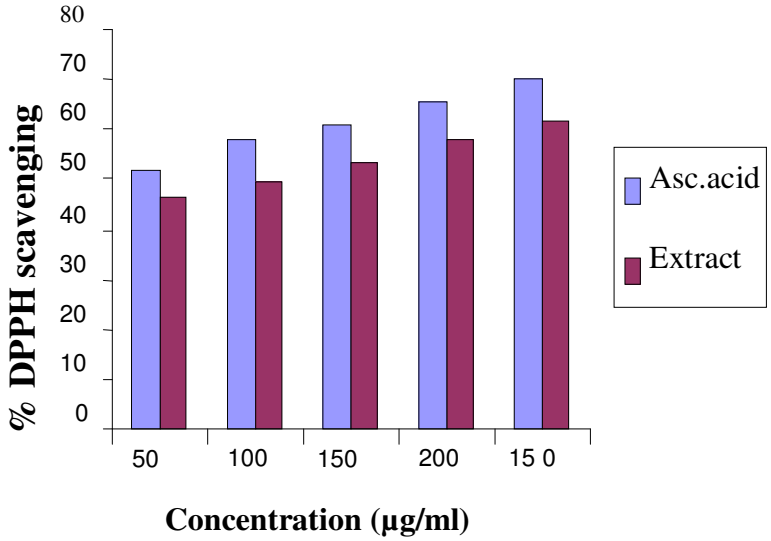

Figure 2. DPPH free radical scavenging of *Mentha longifolia* methanolic extract (MLME).

was measured against brine shrimps growth under controlled condition using normal control is presented in Figures 3 and 4. After complete hatching, shrimps were transferred into glass test tubes already contained saline of sea salt and extract of various concentration of the plant. After 24 h, the effects of extract of different concentration was noted and found that the brine shrimp survival is inversely proportional to the concentration of the plant extract as shown in Figure 3.

From Figures 3 and 4, it is clear that at 500 µg/ml, 45% survival and 55% death occurred. Similarly, at 1000, 1500 and 2000 µg/ml, 40, 20 and 1% survival and 60, 80 and 99% death occurred, respectively. The order/value of LD_{50} of *P. aphylla* methanolic extract is shown below. Figure 3 showed that LD_{50} for this assay is 800 µg/ml and at this value, 50% death occurred, which might be due to

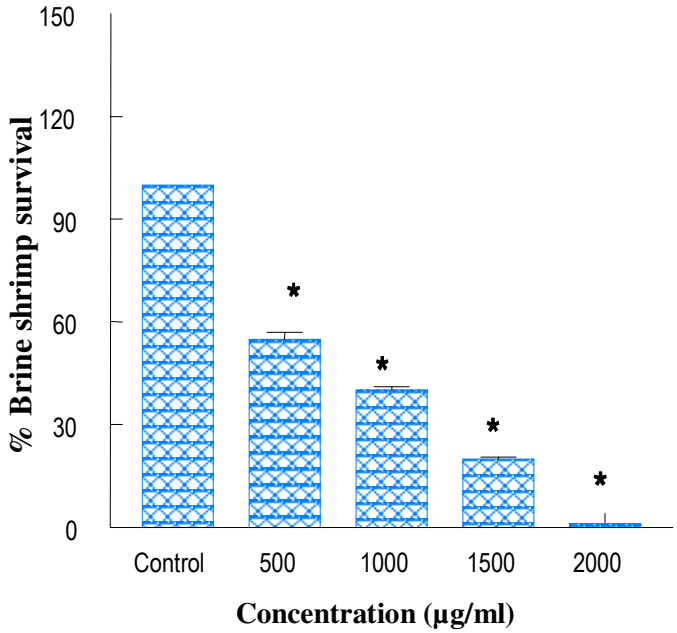

Figure 3. % survival of brine shrimps in various treatments of *Periphloca aphyla*.

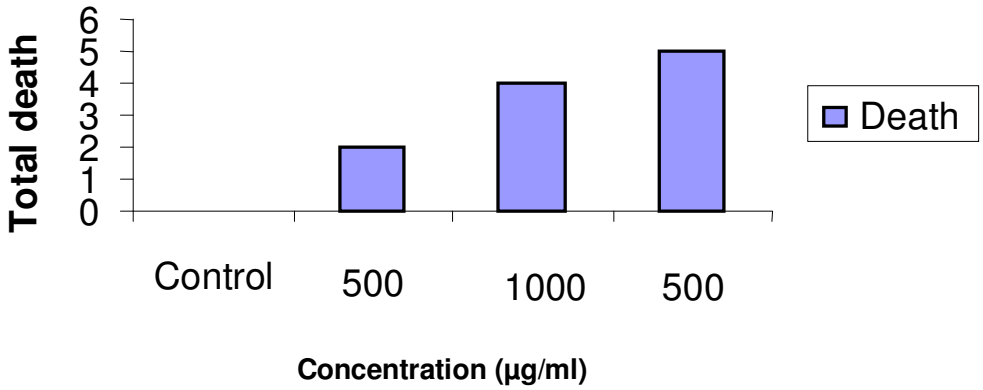

Figure 4. % deaths of *Mentha longifolia* methanolic extract (MLME).

presence of some bioactive toxic constituents. Data revealed that the order of LD_{50} of brine shrimps was recorded. Our results showed that the brine shrimp survival is inversely proportional to the concentration of the extract used. Kanegusuku et al. (2001) reported organic fraction of *Rubus imperialis* (C.) which showed more cytotoxicity. Zaidi et al. (2006) studied that methanolic fraction of *Arceuthobium oxycedri* exhibited 100% cytotoxicity for brine shrimps at high dose which are in accordance with our results. The results of present study evaluated the folk use of these medicinal plants and suggest that methanolic fraction possess some bioactive constituents having anticancer activities that can be the focal point of new drugs having anticancer and protective role against different pathogens. It is clear from Figure 4 that at 500 µg/ml, 75% survival and 25% death occurred. While at 1000, 1500 and 2000 µg/ml, 50, 38.8 and 16.7% survival and 25, 50 and 62.5% deaths occurred, respectively.

Conclusion

Methanolic plant extracts of *M. longifolia* and *P. aphyla* showed significant inhibitory effect, which might be the presence of bioactive phenolic and polyphenolic consti-

tuents in the extract.

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