

**PHARMACOLOGICAL EVALUATION OF ANTIOXIDANT, ANTIMICROBIAL AND PHYTOTOXIC ACTIVITIES OF CRUDE EXTRACT AND PURIFIED COMPOUND OF *LONICERA QUINQUELOCULARIS* HARDW.**

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**Abstract**

In this study crude extract and the pure compound of *Lonicera quinquelocularis* Hardw. plant was studied for antioxidant, antimicrobial and phytotoxic activities. The isolated pure compound showed highest antioxidant activities than the crude extract while the crude extract showed more potential of inhibiting growth of the fungal and bacterial strains as compared to its isolated compound. The DPPH and ABTS scavenging activities of the crude extract and isolated compound was increased with increasing concentrations. Crude extract inhibits the growth of *Aspergillus fumigatus* and *Fusarium solani* 65 and 70%, respectively while the isolated compound showed 22 and 44% inhibition of *A. fumigatus* and *F. solani*, respectively. Both the extract and purified compound also showed growth inhibition of bacterial strain *Salmonella typhimurium*. Moreover, the crude extract and the isolated compound significantly retard growth of maize seedlings.

Medicinal plants have a broad spectrum of applications both in industry and domestic uses due to the bioactive potent compounds. The medicinal properties of plants can be used for 300 different ailments and diseases (Nandakumar 2009). Out of 250,000 species of angiosperms, less than 1% has been screened pharmacologically. Most of the available phyto drugs have been derived from wild resources of plants. In recent times 30% of worldwide drugs are based on natural products isolated from medicinal plants (Grabley *et al.* 1999). Medicinal plants rich in antioxidant phytochemicals have received growing attention as potential chemo preventive agents. Epidemiological studies have demonstrated that antioxidants play a protective role in most of human diseases. Recently the folk medicinal plants are being used as a source to extract new antimicrobial bioactive compounds. A number of allopathic drugs are also comprised of extracts taken from medicinal plants (Rashid and Arshad 2002).

Current studies were designed to explore various biological activities of *Lonicera quinquelocularis* Hardw. crude fractions and isolated pure compounds.

The plant of *L. quinquelocularis* was collected from District Mansehra, in June 2013. The dried plant *L. quinquelocularis* (800 g) was ground to powder and extracted with ethanol at room temperature. The extract was evaporated through rotary evaporate and a thick greenish gummy material was obtained. The material was then dissolved in ethyl acetate and subjected to column chromatography over silica gel (70 - 230 mesh) eluting with *n*-hexane resulting in the isolation of ethyl-3-hydroxy-5-methoxy-4-methylbenzoate. The antioxidant DPPH radical scavenging assay was done according to the modified protocol of Sreejayan (1996). In the assay 1800 µl ethanolic solution of DPPH (1 mM), was mixed with various concentrations (50 to 500 µg/ml) of 200 µl samples in a final volume of 2.0 ml. DPPH solution was mixed with the same above method to

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various concentrations of the ascorbic acid which was used as standard. After 30 minutes' incubation in dark, absorbance was recorded at 517 nm against a blank. The ABTS (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic) decolorization assay was performed following Gyamfi *et al.* (1999) with some modifications. The sample (1200 µl) from each concentration i.e. (50, 100, 150, 200, 250, 500 µg/ml) of samples was mixed with 1800 µl of ABTS solution. Similar process was repeated for ascorbic acid concentration. All test tubes (ethyl acetate soluble fraction, ethyl-3-hydroxy-5-methoxy-4-methylbenzoate, ascorbic acid,) labeled separately were shaken well and incubated in the dark at 25°C for 30 min. Then absorbance was recorded at 734 nm by spectrophotometer after 1 and 10 minutes for each concentration and mean was taken for each reading. The potential to scavenge the ABTS and its radicals was calculated using the following equation:

Antioxidant potentials (%) =  $(A1 - A2/A1) \times 100$ . Where A1 is the absorbance of the control and A2 is the absorbance in the presence of the test sample.

The antimicrobial activities of the samples were analyzed using modified method of Duraipandiyan and Ignacimuthu (2009). Ten mg of each test sample was dissolved in 10 ml DMSO to prepare stock solutions. Terbinafine antifungal agent and streptomycin antibacterial agent were used as standard and were prepared by the same above method. A dilution of 200 µg/ml was prepared from the stock solution for both the samples and standard using DMSO as diluting solvent. DMSO was used as a negative control. Sabouraud Dextrose Agar (SDA) media was used for fungal growths and nutrient agar was used for bacterial strain culture. 100 µl of each sample was added to the test tubes, containing the active culture of the fungus and bacterial strains i.e. *A. fumigatus*, *F. solani* and *Salmonella typhimurium*. All the test tubes were packed air tightly and were placed in incubator at 37°C for 7 days. Fungus growth in the test tube was measured after 10 days and percentage growth inhibition was calculated.

Phytotoxic assays were performed using modified protocol of McLaughlin (1988). 1 mg/ml concentration stock solution was prepared in H<sub>2</sub>O for all the testing samples. Further dilutions of 500 and 100 µg/ml were prepared from stock solutions for all samples. For each concentration of each sample 4 Petri plates were used one plate in each of the four plates was control that contained distilled water and the remaining three contained the above solutions of the extract. Filter paper was set in each plate and 3 ml solution of each concentration of every sample was sprayed on the filter paper in plate. Fresh seeds of maize soaked in distilled water were sowed in each plate. In each plate 6 seeds were sowed. In order to check the phytotoxic effects of the tested samples, radical and shoots of the maize seedling were measured after 10 days and average means were taken.

The crude extract of *L. quinquelocularis* and isolated compound were studied for its biological activities. Antioxidant activities were determined through ABTS and DPPH free radicals scavenging assays. Various concentrations of the crude extract (50, 100, 150, 200, 250, 500 µg/ml) and the pure compound showed significant scavenging activity compared with ascorbic acid used as a standard. Results showed that the free radicals scavenging activities increasing with increasing the samples concentrations (Fig. 1).

Similar experiments for DPPH radical scavenging assay were designed. The crude extracts and the isolated compound showed good scavenging activities against DPPH free radicals (Fig. 2).

The crude fraction and pure compound ethyl-2-hydroxy-4-methoxy-3-methylbenzoate retard the maize seedling growth (Fig. 3). The crude extract and isolated compound showed growth inhibition effect on fungus and bacterial strains.

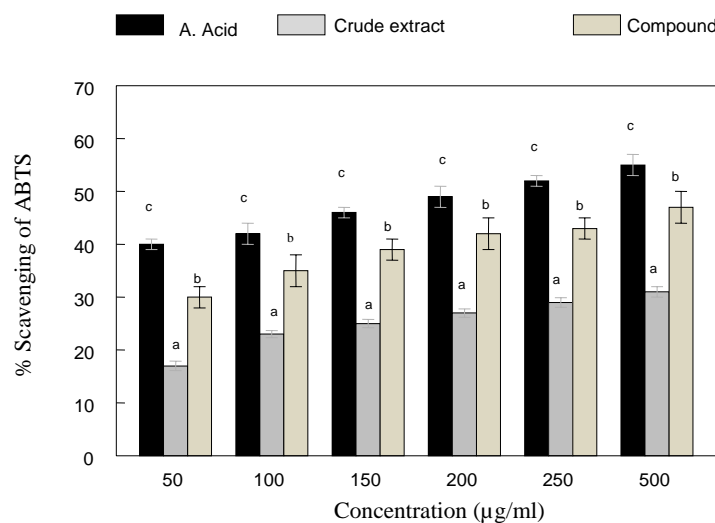


Fig. 1. ABTS scavenging activities of various concentrations of ascorbic acid and samples. Data are mean values of different experiments run in duplicate ± SEM.

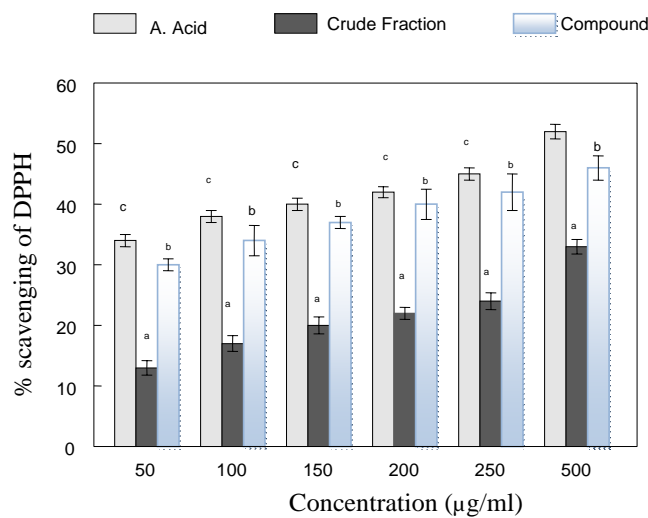


Fig. 2. DPPH. scavenging activities of various concentrations of ascorbic acid and samples. Data are mean values of different experiments run in duplicate ± SEM.

In the present study, pharmacological activities of ethyl acetate crude extract of *L. quinquelocularis* and the compound isolated from this extract (ethyl-3-hydroxy-5-methoxy-4-methylbenzoate) was investigated. The isolated pure compound showed highest antioxidant activities against DPPH and ABTS radicals than the crude extract. While in case of antimicrobial and phytotoxic effects, the crude extract showed higher activities than the isolated pure compound. The results indicate that *L. quinquelocularis* possesses antimicrobial activities (Shahnoor *et al.* 2013). The crude extract and isolated compound showed growth inhibition effect on fungus and bacterial strains (Fig. 4).

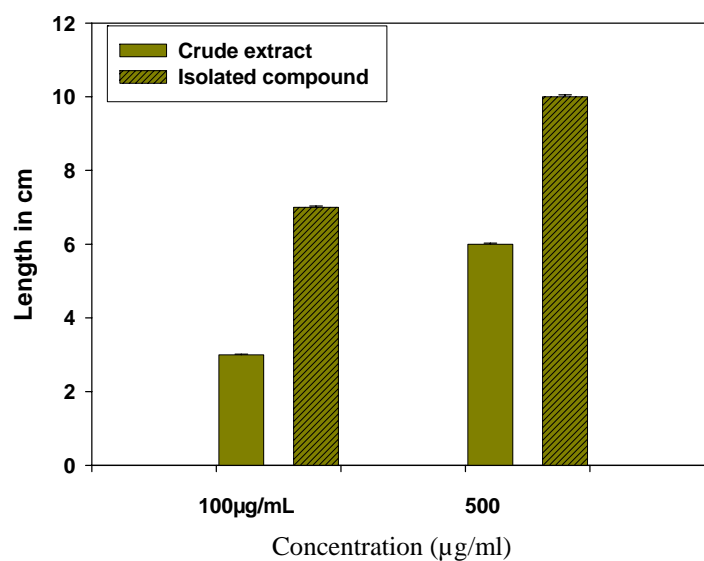


Fig. 3. Phytotoxic effects of *L. quinquelocularis* of the crude extract and purified compound against rice growth after 10 days' treatment. Data are the mean of different values of three different experiments run in duplicate.

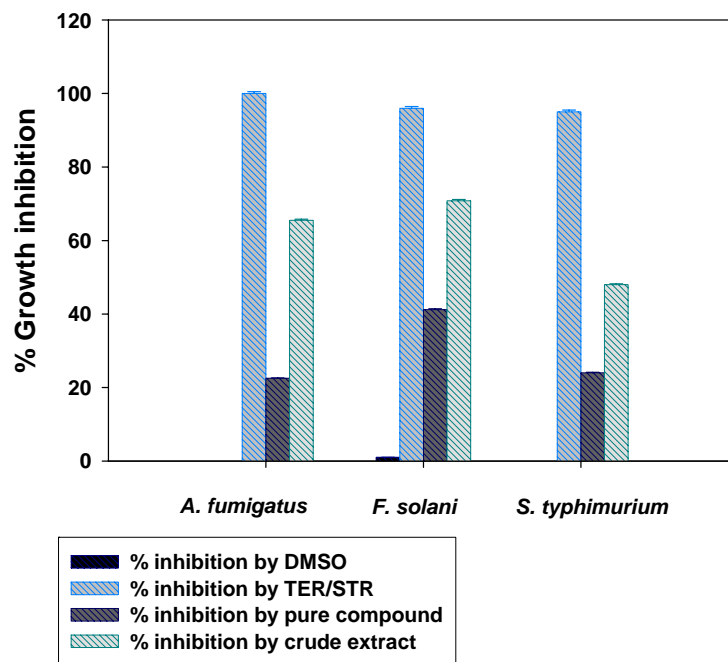


Fig. 4. Antimicrobial activities of the crude extract and pure compound of *L. quinquelocularis*. Data are the averages of different values from different experiment run in duplicate  $\pm$  SEM.

Results demonstrated that *L. quinquelocularis* is a rich source of important pharmacological bioactive substances. Therefore, this plant can be a best candidate against different disease caused by free radicals and microorganism. Furthermore, the bioactive constituent present in this plant can work as herbicide.

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### References

- Duraipandiyan V and Ignacimuthu S 2009. Antibacterial and antifungal activity of Flindersine isolated from the traditional medicinal plant, *Toddaliaasiatica* (L.) Lam. J. Ethnopharmacol. **123**: 494-498.
- Grabley S and Thiericke R. 1999. Bioactive agents from natural sources: trends in discovery and application. Adv. Biochem. Eng. Biotechnol. **64**: 101-54.
- Gyamfi MA, Onamine MY and Aniya Y 1999. Free-radical scavenging action of Medicinal herbs from Ghana: *Thonningiasanguinea* on experimentally-induced liver injuries. Gen. Pharmacol. **32**: 661-667.
- McLaughlin JL. 1988 Bioassay for discovery of antitumoral, antiviral agents from natural sources: In: Proceedings of NIH Workshop Bethesda. pp.22
- Nandakumar J 2009. *Ex situ* conservation of medicinal plants in Valikamam area of the Jaffna Peninsula, Sri Lanka. New Biot. J. **25**: 370.
- Rashid A and Arshad M 2002. Medicinal plant diversity, threat imposition and interaction of a mountain people community. In: Proceeding of Workshop on Curriculum Development in Applied Ethnobotany. Published by the Ethnobotany Project, WWF Pakistan, 34-D/2, Sahibzada Abdul Qayyum Road Peshawar, Pakistan. pp. 84-90.
- Shahnoor, Khan DF, Ahmed M, Khan RA, Khan MI and Khan S 2014. Determination of antifungal and phytotoxic activities of three different fractions of *Lonicera aquinquelocularis* (Translucent Honeysuckle) plant. Journal of Pharmacognosy and Phytochemistry **2**(6):167-172.
- Sreejayan M 1996. Free radical scavenging activity of curcuminoids. Arzenium-Forsch. Drug Research **6**:169-171.
- Tepe B, Daferera D, Sokmen A, Sokmen M. and Polissiou, M. 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chem **90**: 333-340.
- Uttara AV, Singh P, Zamboni Pand Mahajan RT. 2009. Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic option. Curr. Neuropharmacol. **7**(1): 65-74.

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