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Short Communication

CYTOTOXIC, ANTIOXIDANT, ANTIMICROBIAL ACTIVITIES OF METHONOL CRUDE EXTRACTS OF *CARDIA OBALIQUA* (LINN.)

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ABSTRACT

Cardia obaliqua was used traditionally in Pakistan for the treatment of diabetes, microbial infection and jaundice. In the present study we arranged to evaluate the antimicrobial, antioxidant and cytotoxic activities of *Cardia obaliqua* methanol extract. *Cardia obaliqua* was obtained from Surrani, District Bannu for preparation of crude methanol extract. A standard protocol of agar well diffusion method was used for characterization of various bacterial strains. Agar dilution method was subjected to investigate the antifungal activities. Death rate of brine shrimps was checked to investigate cytotoxic efficacy of the crude extract. The results showed that the crude extract significantly controlled the microbial growth as compare to control, which was in agreement with the spacious use of tested plant samples in primary healthcare center. Similarly the extract showed significant potency in scavenging of free radicals. From the present study it is inferred that *Cardia obaliqua* methanol extract was act as a vital role for the detection of new antimicrobial drugs and antioxidant agents; which confirm its conventional uses and its management as beneficial mediator.

Key words: *Cardia obaliqua,* Methanolic extract, Cytotoxic activity, Antioxidant activity, Antimicrobial activity.

INTRODUCTION

In the recent age human being throughout the globe is suffering from various infections which cause a large number of diseases. To overcome such miserable condition various strategies are being in process. Among them medicinal plants play crucial role in care the improvement of human health (Bruneton, 1955). In this regard ethano medical knowledge of traditional medicinal plants and their pharmacological screening are in practice in various regions of Pakistan (Khan *et al*., 2016). Medicinal plant contains a variety of potent bioactive ingredients which provide control and immunity to living things against various harmful agents (Khan *et al*., 2016). Medicinal plant contains a large number of antioxidants compounds which are used for the prevention of oxidative stress and protract the life of the functional substance. For this purpose in the present, it is decided to add the antioxidants in suitable amount in the food as an additive, in cosmetics and in pharmaceutical products (Sahreen *et al*., 2015). According to different reports it was shown that various parts of medicinal plants viz; fruits, leaves, oil seed, roots and vegetables have free radical scavenging and microbial growth control efficacy (Rababah *et al*., 2004) which may be used as natural antioxidants and antibiotics (Sahreen *et al*., 2014). *Cordia obaliqua* plant are found worldwide but mostly in warmer regions of the world, producing edible fruits, known as a clammy cherries, glue berries, sebesten, or snotty gobbles and lasora. The fruits are also used as a vegetable and pickle. *Cordia obaliqua* is used as an ornamental plant and its wood is commercially harvested. According to binomial nomenclature *Cordia obaliqua* belong to Boraginaceae. *Cardia oblica* is a local plant of District Bannu which is commonly used for the control of various human ailments without pharmacological confirmation. The present project was basically designed for the pharmacological evaluation via different assays viz; antioxidant, cytotoxic and antimicrobial activities of *Cardia oblique* crude methanol extract.

MATERIALS AND METHODS

Cardia obaliqua fresh leaves were obtained from Landidak area of District Bannu during fruiting sessions. After shad drying of fresh parts at room temperature (25^0C), it was ground mechanically to get fine powder. Among powder, 1 kg of *Cardia obaliqua* fine powder was socked in 70% methanol (1.5 L) and shacked randomly. After 96 hrs, the extract was filtered via Whatman filter paper No.1. Filtrate was concentrated using rotary evaporator at 38°C to get methanol crude extract (COME) and was kept at 4°C in the refrigerator for further in vitro investigation.

Cytotoxic brine shrimp lethality test was carried out according to standard protocol with some modification as described by Khan *et al.,* (2015) for the determination of cytotoxic activity. DPPH (1, 1-diphenyle -2- picryle hydrazyl) scavenging activity was conducted using the procedure of Gymafi *et al*., (1999) to show antioxidant efficacy of crude methanol extract. To check the antifungal activity of the methanol crude extract of *Cardia obaliqua*, the standard protocol of Duraipandiyan and Lgnacimuthu (2009) was used.

Antibacterial activity of *Cardia obaliqua* methanol crude extract was carried out by the protocol of Bagamboula *et al*., (2003).

RESULTS

Prime screening of the extract through cytotoxic activity provides useful information about the antitumor and anticancer activity for the future use of the medicinal plants extract. Cytotoxic effect of the *Cardia obaliqua* methonolic crude extracts was measured and noted against brine shrimps growth as shown in Table 1. From the Table 1, it is clear that 50µg/ml treatment of crude extract showed 90% survival and 10% death, however at 100, 250, 500 and 1000 (µg/ml), there was 70, 50, 30 and 20 (%) survival and 30, 50, 70 and 80 (%) death occurred respectively which may be due to the presence of bioactive constituents present in the plant extract.

1,1- diphenyl 2- picryl – hydrazyl (DPPH) have the aptitude to obtain electrons from the antioxidants, that is why it is broadly utilized for the in vitro antioxidants scavenging assays of the medicinal plants. Table 2 shows the % scavenging activities of COME and ascorbic acid against the DPPH free radicals. By using the crude methanol extract with the increasing concentration such as 50<100<150<500<1000 (µg/ml), significant scavenging activities were observed viz; 54.6±2.6<58.0±3.1<65.5±2.9<77.9±3.8<89.1±3.4. Similarly ascorbic acid showed potent DPPH free radicle scavenging activities which was used as a standard.

Cardia obaliqua methanol crude extracts revealed low activity (29.78 %) against *Aspergillus fumigatus* followed by *Aspergillus flavus* (38.60%) while the highest activity was shown against *Aspergillus niger* (49.44%) and *Fusarium salmoni* (45.49%) as shown in Table 4. Similarly the terbinofine, a positive control has indicated high activity against these fungal strains. Terbinofine shows low activity (84.38%) against *Fusarium salmoni* followed by *Aspergillus Flavus* (85.73%) in comparison highest activity was found against *Aspergillus niger* (90.50%) and *Aspergillus fumigatus* (87.23%). DMSO (negative control/normal control) indicates zero percent (0%) inhibition activity against all the used four fungal strains.

COME showed low activity (13±1.5mm) against *Escherichia coli* followed by *Staphylococcus aureus* (16±2.4mm). The methanolic extract exposed highest antibacterial activity against *Micrococcus luteus* (21 mm) while comparatively high activity was observed against *Pseudomonas aeruginosa* (17±2.3 mm) as shown in Table 5. Highest activity was observed against *Staphylococcus aureus* (21±1.4) and in comparison high activity was observed against *Escherichia coli* (17±2.7 mm), while highest activity was observed against *Micrococcus luteus* (23±2.0 mm) and comparatively high activity was found against *Pseudomonas aeruginosa* (21±1.8 mm) as shown in Table 5.

Table 1. Effect of *Cardia obaliqua* methonolic against brine shrimp assay.

Concentration of plant extract	Total No of brine shrimps	Survived brine shrimps	% Survival	% Death
50µg/ml	10	9	90	10
100µg/ml	10	7	70	30
250µg/ml	10	5	50	50
500µg/ml	10	3	30	70
1000µg/ml	10	2	20	80

Mean± SEM

Table 2. % DPPH free radical scavenging activity

Concentration (in µg/ml)	%COME Scavenging	% Ascorbic acid Scavenging
50	54.6±2.6	63.2±2.5
100	58.0±3.1	67.1±2.8
150	65.5±2.9	71.6±3.9
500	77.9±3.8	93.9±4.6
1000	89.1±3.4	97.1±5.0

Mean± SEM

Table 3. % ABTS free radical scavenging activity

Concentration (in µg/ml)	%COME Scavenging	% Ascorbic acid Scavenging
50	35.17±2.1	46.25±2.1
100	42.11±2.3	51.65±4.1
150	55.06±1.7	63.20±3.7
500	69.77±1.8	85.10±2.5
1000	80.35±2.4	95.96±3.1

Mean± SEM

Table 4. Antifungal activity of *Cardia obaliqua* methanolic extracts.

Sample	Antifungal activity in term of percentage (%) inhibition			
	Aspergillus niger	***fusarium salmon***	***Aspergillus Flavus***	***Aspergillus fumigatus***
COME (200 µg/ml)	49.44±2.5	45.49±6.1	38.60±3.4	29.78±2.0
Terbinofine (200 µg/ml)	90.50±2.1	84.38±5.1	85.73±5.6	87.23±5.1

Mean± SEM

Table 5. Antibacterial activity of *Cardia obaliqua* methanolic extracts (COME)

	Micrococcus luteus	***Escherichia coli***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***
Concentration (mg/ml)	Zone (mm)	Zone (mm)	Zone (mm)	Zone (mm)
1 mg/ml cefix	16±1.9	27±2.4	18±2.0	29±2.4
1 mg/ml Ampicil	23±2.0	17±2.7	21±1.8	20±2.3
1 mg/ml COME	20±1.2	19±1.5	15±1.6	14±1.7
3 mg/ml COME	22±1.3	18±1.9	14±2.1	13±1.8
5 mg/ml COME	21±1.4	13±1.5	17±2.3	16±2.4

Mean± SEM

DISCUSSION

Reactive oxygen species (ROS) in lesser quantities are useful for the normal metabolism and signal transduction but in large amounts produces oxidative stress which outbreaks and harm numerous units like protein, lipids and DNA (Halliwell and Gutteridge, 1999). Medicinal floras showed crucial role in the cure of diverse mortal diseases viz; free radicals stress, inflammatory disorder, cardiac failure and metastasis in the biosphere from very long time. Diverse portions of natural products are recycled frequently for the handling of numerous sicknesses comparatively to the drugs prepared in various pharma industries. Pakistan is a rich state in therapeutic floras which are locally used in folk medicines for the treatments of various diseases like infections, cardiovascular diseases, diseases of digestive system and skin by local healer (Khan *et al*., 2016). The results got from the current study of *Cardia obliqua* (Linn) methanol extracts display important scavenging efficacy. The antioxidant activity of the methanol extracts of the medicinal florae is due to the phenolic and polyphenolic compounds present in them, which distinctly decrease the free radicals (Shah *et al.,* 2017). Our findings reveals some resemblances with the examination of Hagerman *et al*. (1998) and Falleh *et al*. (2008) who stated that free radicals are distinctly scavenge by medicinal plants. The consequences got from our trials are also stayed by the conclusions of Duenas *et al*. (2006) and Kilani *et al*. (2008). As fungi yield mycotoxin which meaningfully affect human health. Some fungus abolishes our foods and interrupts their dietetic value thus made them useful for our routine (Sun *et al*., 2002). In our current study the antifungal activity of *Cardia obliqua* (Linn), confirmed that fungal strains are considerably repressed by them. Numerous reports of the medicinal floras for microbial actions designated that their actions are due to the occurrence of phenolic compounds and saponins (Aboaba and Efuwape 2001, Baydar *et al*., 2004, Rodringuez *et al*., 2007; Mohanta *et al*., 2007).

REFERENCES

Aboaba, O. O. and B. M. Efuwape (2001). Antibacterial properties of some Nigerian species. Bio. Res Communi. 13: 183–188.

Bagamboula CF, M.Uyttendaele and J Debevere (2003). Antimicrobial effect of spices and herbs on *Shigella sonnei* and *Shigella flexneri*. J. Food Prot. 66: 668–673.

Baydar NG, O. Sagdic, G. Ozkan and S. Cetin (2006). Determination of antibacterial effects and total phenolic contents of grape (*Vitis vinifera* L.) seed extracts. *Intl. J. Food Sci. Technol.* 41: 799–804.

Bruneton J. (1955). Pharmacognosy, phytochemistry, Medicinal plants. France: Lavoisiler publishing co. 265–380.

Duenas M, T. Hernandez and I. Estrella (2006). Assessment of *in vitro* antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. Food Chem. 98: 95–103.

Duraipandiyan V. and S. Ignacimuthu (2009). Antibacterial and antifungal activity of Flindersine isolated from the traditional medicinal plant, *Toddalia asiatica* (L.) Lam. J. Ethnopharmacol. 123: 494–498.

Falleh H, R. Ksouri K. Chaieb N. Karray-Bouraoui, N. Trabelsi, M. Boulaaba. and C. Abdelly (2008). Interspecific variability of antioxidant activities and phenolic composition in Mesembryanthemum genus. Food Chemical Toxicol 47: 2308–2313.

Gyamfi MA, M.Yonamine and Y. Aniya, (1999). Free radical scavenging activity of medicinal herb of Ghana: *Thonningia sanguinea* on experimentally induced liver injuries. General Pharmacol. 32, 661–667.

Hagerman A.E, K.M. Riedl, GA. Jones K.N. Sovik, N.T. Ritchard. and P.W. Hartzfeld (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem. 46: 1887–1892.

Halliwell B. and J.M.C. Gutteridge (1999). Free radicals in biology and medicine. Oxford University press; 617–783.

Khan A, R.A. Khan and M. Ahmed (2015). In vitro antioxidant, antifungal and cytotoxic activity of methanol extract of *Calligonum polygonoides*. *Bangladesh* J. Pharmacol. 10: 316–320.

Khan W, RA Khan, Ahmed M, Khan LU. and Khan MA: 2016. Pharmacological evaluation of methanol extracts of *Cyperus scariosus*. Bangladesh J. Pharmacology 11: 353-58.

Kilani S, MB Sghaier, I Limem, I Bouhlel, J Boubaker, W Bhouri, I Skandrani, A Neffatti, RB Ammarb, MG Dijoux-Franca, K Ghedira and L Chekir-Ghedira (2008). *In vitro* evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of *Cyperus rotundus*. *Bio Res. Technol.* 99: 9004–9008.

Mohanta TK, J.K. Patra, S.K. Rath, D.K .Pal. and H.N. Thatoi. (2007). Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semicarpus anacardium* L.f. Sci. Res. Assay 2: 486–490.

Rababah T.M, E.L. Rice, W.S.Hettiarachy and R. Horax. (2004). Total phenolics and antioxidantactivities of fenugreek, green tea, black tea, grap seed, ginger, rosemary, gotu, kola, ginkgo extracts, vitamin E, and terbutyl hydroquinone. J. Agric. Food Chem. 52: 5183–5186.

Rodriguez N, Amils R, Jimenez-Ballesta R, Rufo L. and Dela-Fuente V: 2007. Heavy metal content in Erica andevalensis: an endemic plant from extreme acidic environment of Tinto River and its soils. *Arid Land Research Management* 21: 51–65.

Sahreen S, R.A.Khan and M.R. Khan (2015). Evaluation of phytochemical content, antimicrobial, cytotoxic and antitumor activities of extract from *Rumex hastatus* D. Don roots. BMC Compl. Alter. Med. 15:211; DOI 10.1186/s12906-015-0736-y.

Sahreen S, M.R. Khan and R.A. Khan (2014). Estimation of flavoniods, antimicrobial, antitumor and anticancer activity of *Carissa opaca* fruits. BMC Compl. Alter. Med. 13**:**372.

Shah AU, R.A. Khan. M.Ahmed (2017). Pharmacological activities of methanolic crude extract of *Quercus dialatata* fruit. Bangladesh J. Pharmacol. 12: 91-92.

Sun J, Y. Chu X.Wu and R. Liu (2002). Antioxidant and anti-proliferative activities of common fruits. J. Agric. Food Chem. 50 (25): 7449–7454.