Pak. J. Bot., 49(3): 1161-1165, 2017.

PHYTOCHEMICAL SCREENING AND BIOLOGICAL ACTIVITIES OF *TRIGONELLA INCISA* AND *NONEA EDGEWORTHII*

SALIM UR REHMAN[1], RIZWAN FAISAL[2], ZABTA KHAN SHINWARI[3*], NISAR AHMAD[1] AND IJAZ AHMAD[4]

[1] Department of Botany, Kohat University of Science & Technology, Kohat, Pakistan
[2]Rehman Medical College, Peshawar, Pakistan
[3]Department of Biotechnology, Quaid-i-Azam University, and Pakistan Academy of Sciences, Islamabad, Pakistan
[4]Department of Chemistry, Kohat University of Science & Technology, Kohat, Pakistan
[*]Corresponding author's email: shinwari2008@gmail.com

Abstract

The extracts and its derived fractions from two important medicinal plants species *Trigonella incisa* and *Nonea edgeworthii* were tested for biochemical potential. The aim of our research was to encourage drug finding work with plants. The crude extract and fractions from *Trigonella incisa* plant were found to be most potent against *Pseudomonas aeuriginosa* as compared to *Escherichia coli* and *Salmonella typhi.* The Chloroform fraction showed outstanding inhibition 11 mm against *Pseudomonas aeuriginosa* followed by crude extract, *n*-butanol and aqueous fractions each giving 9 mm inhibition. The *n*-butanol fraction of *Trigonella incisa* revealed 8 mm inhibition against *Escherichia coli* second by aqueous fraction with 7 mm inhibition. Moderate inhibition (8 mm) was showed by crude extract and chloroform fraction against *Salmonella typhi*. In case of *Nonea edgeworthii* plant aqueous and ethyl acetate fraction were found to be most active against *Pseudomonas aeuriginosa* and *Escherichia coli* giving inhibition of 14 mm each which is found to be best inhibition even more than the inhibition showed by antibiotic used. Crude extract and chloroform fraction of the plant showed 12 mm and 10 mm inhibition against *Salmonella typhi.* Both the selected plants were found equally potential against the tested fungi. n-hexane and chloroform fractions of *Trigonella incisa* give 10 mm inhibition against *Fusarium oxysporum* and *Alternaria alternata* respectively while crude extract from *Nonea edgeworthii* give 11 mm inhibition against *Alternaria alternata*. Over all poor scavenging activity was showed by selected plants. Ethyl acetate fraction of both plants was found to be reasonably good when compared with standard. The low antioxidant profile of the plants may be due to the absence of flavonoids in plants .In preliminary phytochemical screening alkaloid, phenol and saponins were reported in both plants.

Key words: Medicinal plants, Antibacterial activities, Antifungal activities, Antioxidant activities, Phytochemical screening, Drug sighting.

Introduction

Trigonella incisa Linn belongs to family Fabaceae. The genus *Trigonella* is represented by 70 species and is distributed in Mediterranean zone. In Pakistan there are 16 species of *Trigonella*. It is an annual herb. Leaves are pinnately trifoliolate. Leaflets are usually dentate. Stipules adnate to petiole. Stem is prostrate and branched. Root is tape. Inflorescence is spike. Bracts are minute and bracteoles are absent. Calyx teeth are equal or unequal. Corolla is yellow free from the staminal tube. Stamens are diadelphous, 9+1, anthers uniform. Ovary is sessile and ovules are numerous, style glabrous, stigma terminal. Fruit are linear, dehiscing along one suture or indehiscent, continuous within, 1-many seeded (Nasir & Ali 1977). *Trigonella incisa* is therapeutically utilized as antiviral, anti inflammatory and as a hunger stimulator (Esmaeili *et al.*, 2012). *Nonea edgeworthii* Linn belongs to family Boraginacae. There are 55 species of genus *Nonea* and in Pakistan the genus is represented by8 species. It is annual herb up to 9-40 cm or more tall in height. Stem is hairy, erect and branched. Leaves are hairy, lanceolate basal and cauline leaves 35-100 x 5-15 mm, hairs similar to those on stem and branches. Petioles of cauline and basal leaves are winged. Root is tape root. Inflorescence elongated in fruit, short otherwise. Flowers are subsessile. Pedicels pubescent, up to 4 mm in fruit. Calyx 6-7 mm long, up to 10 mm in fruit. Corolla creamy white, tube 5-6 mm long. *Nonea edgeworthii* is mostly found as weeds and are distributed in the plains and hilly areas of Pakistan and India (Ali & Qaiser 1993). The plant is medicinally utilized for the treatment of cough, lungs growth, respiratory disruption and in microbial infections (Matin *et al.*, 2001; Shinwari et al. 2006).

Materials and Methods

Plant collection: The selected plants *Nonea edgeworrthi* and *Trigonella incisa* were collected from district Karak Khyber Pakhtunkhwa, Pakistan. The plants were identified with the help of literature and the plant specimens were kept in herbarium department of Botany KUST with voucher No Bot. 441 and Bot. 442. Fresh specimens of the plants were taken and were cleaned from dust and sand particles through tape water. Plants were dried at room temperature i.e. 25 c° and through grinder each plant specimen was turned into fine powder and latterly placed in a bags for further examinations.

Crude extracts preparation: 4Kg of plant powder was taken and then it was soaked in 8L analytical grade methanol. This mixture was regularly blended after every 24hrs for the period of 15 days. The solvent having the extract was then cleaned with a Muslin cloth in order to get the percolate. The percolate was then moved by rotator evaporator and the temperature was increased up to 55 c° beneath less pressure to evaporate solvent. The extract was later dried thoroughly and got a greenish color

crude. This was further suspended in distilled water and partitioned successively with n-hexane, chloroform, ethyl acetate and n-butanol to obtain n-hexane-soluble, chloroform-soluble, ethyl acetate-soluble, n-butanol-soluble and aqueous fractions, respectively.

Media preparation: First in nutrient agar, the bacterial strains were replenished by keeping it for 12hrs in incubator. Then, in conical flask, 7g of nutrient agar was adopted and up to 250ml of purified water was mixed to it. The flask was given a moment for some time in order to make the mixture of it, it was tightened with cotton and placed in autoclave and heated up to 121c° for duration of 15 min in order to sterilized.

Test micro organisms: In this research, three strains of bacteria i.e., *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* and two strains of fungi i.e. *Alterneria alternata* and *Fusarium oxysporum* were applied. The particular strains were taken from the Department of Microbiology KUST Kohat.

Methodology of experiments for antibacterial and antifungal activities: For this experiment, agar well diffusion method was used. The crude extract and its derived fractions were diluted in Dimethyl Sulfoxide (DMSO). Petri plates of 9cm diameter were taken and the nutrient media was putted in it and total of 6 wells were made in each plate. The bacteria were spreads on the surface of Petri plates with sterile swabs. The relevant extract and fractions were put in each well. Cefotaxime which is an antibiotic was used as standard. The plates were incubated in incubator and heated up to 37c for the period of 24hrs/48hrs. The actions of the pathogens were examined by calculating the inhibition zones. All the tests were repeated three times to minimize the error (Mahesh & Satish, 2008).

Antioxidant activity: α, α -- diphenyl – β – Picryl – Hydrazyls (DPPH) Radical Scavenging is a free stable radical and it is largely used in order to examine the radical scavenging activity of the antioxidant compounds. This technique is generally based on the diminution of DPPH in the solution of methanol in the availability of hydrogen which donates antioxidant because of the formation of non radical form DPPH – H. Because of this transformation change occur in the color from purple to yellow, which is measured through spectrophotometer. When this change occurs then the absence of the purple color is observed at 517nm. By using 1, 1 diphenyl – β – Picryl – Hydrazyl or 2-2 diphenyl – β – Picryl – Hydrazyl is calculated the free radical scavenging through the method of Johns and McCune (2002). It is placed in incubator for the period of 10 minutes in darkness and then the absorbance is calculated at 517nm (Alagumanivasagam *et al.,* 2012). In this method Trolox was used as positive control. The inhibition percentage can be measured by using the following formula.

Inhibition Percentage = a0 – a1 x 100.

In which a1 is the absorbance of test and a0 absorbance of control

Phytochemical analysis: Qualitative phytochemical examination was performed in order to examine the phenols, saponins, flavonoids, glycosides, alkaloids and terpenes in the unrefined powder of selected plants (Ogunyemi, 1979).

Results and Discussions

Traditional and native medicines, making of new drugs, ethno-botany are some areas which are always the field of interest of medicinal plant research (Shinwari et al. 2009; Qasim et al. 2010). From ancient times, natural compounds are the greatest source of lead molecules and play an important role in the development and making of new drugs and these natural products and those products which we get from them have been developed for clinical purposes and also for pharmaceutical uses (Shinwari, 2010; Shinwari and Qaiser, 2011). The structural characteristics and stereo chemical characteristics of the natural plants make them important in order to explore new compounds (Aqil *et al*., 2006). Plant extracts and pharmaceutical activities of major phytocompounds such as fatty acids, terpenoides, phenolic, carotenes, alkaloids, flavonoids, and tannins create fractions (Dewick, 2002; Hussain *et al*. 2011; Siddique *et al*. 2014).

Antibacterial activity: For present studies we have selected two plants *Trigonella incisa* and *Nonea edgeworthii* to examine its pharmacological and phytochemical potential. Methanol extracts and its derived fractions were obtained from each plant. In microbicidy results it was noted that chloroform fraction of *Trigonilla incisa* was most active against *Pseudomonas aeruginosa* giving 11 mm inhibitionwhile crude extract and chloroform fraction showed 8mm inhibition each against *Salmonella typhi*. The *n*-butanolfraction of *Trigonella* brings out 8mm inhibition against *Escherichia coli* second by aqueous fraction with 7mm inhibition. *Trigonilla incisa* plant was found to be least active against *Escherichia coli* (Table 1). To execute the biological activity of samples of different plants, the ethno botanical approach provides strong clues. This approach gives us a high percentage of constructive results which is the assurance of the biological activity. The results which we have got from the present study proved that many bioactive compounds such as phenolic and alkaloids which were reported in the phytochemical screening may have inclination towards the antimicrobial prospective. Antibacterial and antifungal potency of phenol is the center of study of different writers (Bruneton, 1999; Walter et al. 2011). To make composite with bacterial cell wall, antimicrobial activity can be assigned to plant bioactive compounds which limit the range of the microbial growth (Kuete *et al.,* 2006). Our present study paves the way for the use of bioactive fractions from the plants which are tested in order to treat the infections related with the selected *microorganisms*. In the antibacterial profile of Nonea edgeworthii the most active fraction was ethyl acetate which showed 14mm of inhibition against *Pseudomonas aeruginosa* and *Escherichia coli*. These

inhibition zones are noted to be the best inhibitions even more than the inhibition showed by standard antibiotic used. Crude extract and chloroform fraction of the plant showed 12mm and 10mm inhibition against *Salmonella typhi*. Chloroform fraction of *Nonea edgeworthii* plant revealed 9mm inhibition against *Pseudomonas aeruginosa* (Table 2). It may be because of having the fatty acid esters and alkaloids that we have performed antibacterial activity of the selected plants (Gohar *et al.,* 2010). Those plants in which there is fatty acid ester in their extract, are according to [Preethi *et al.,* 2010], strong and powerful antimicrobial fractions has also explained some same type of finding in the presence of marine antibacterial agents, where hixadeconic acid and other such factors which we get from marine bacteria were separated and noted out the potential of these antibacterial different pathogens. Both of them seen the antibacterial activity of the separated compounds but they also observed that the activity of raw ethanol extract was more than the activity of isolated compounds.

Antifungal activity: In our environment, we found fungi everywhere and most of the common infections are due to the fungal pathogens (Lopes and Martins, 2008). While doing research in laboratory, several works showed that many plant tissues such as seed, roots, flowers and leaves has great inhibitory properties against fungi (Davicino *et al.,* 2007). During our present study, the selected medicinal plants *Trigonella incisa* and *Nonea edgeworthii* were tested against to two fungal strains. Both the selected plants were found equally potential against the tested fungi. N-hexane and chloroform fraction of *Trigonella incise showed* 10mm inhibitions against *Fusarium oxysporum* and *Alterneria alternata* respectively while crude extract from *Nonea edgeworthii* gave 11mm inhibition against *Alterneria alternata* (Tables 1, 2). From different areas of the world, antimicrobial characteristics of plant extract have been noted having increasing frequency (Cowan, 1999). In order to control phytopathogenic fungi, synthetic fungicides are used, but use of these is strictly prohibited because of the dangerous consequences of poisonous chemicals on the environment and human health. (Harris *et al.,* 2001; Gilani *et al*., 2010)

Antioxidant activity: The non-enzymatic method which is used generally in order to furnish the foremost information is DPPH test which is based on the ability of extracts to scavenge free radicals. In our present study the plants which we have tested were found to have very low antioxidant profile. Overall poor scavenging activity was showed by *Trigonella incisa* and *Nonea edgeworthii* plants. Ethyl acetate fraction of both plants was found to be reasonably good when compared with standard. The plants have little antioxidant profile which may be due to presence of lesser amount of flavonoids in these plants (Table 3). The greatest source of the biologically active compounds is the medicinal plants which are used as raw material for many centuries for treating various diseases (Borris, 1996). Previously scientists have evaluated twelve medicinal plants in order to perform the free radical scavenging activity by using DPPH radicals. Out of these tested plants, 7 plants were found having more than 70% scavenging activity (Couladis *et al.,* 2003). Many Vitamins, phytochemicals and minerals may be the source of protection against such destructions which are caused by ROS. From different researches, it has been proved that plants are the source of powerful antioxidants and these plants also represent that they are the source of natural antioxidants (Es-Safi *et al.,* 2005).

Table 1. Microbicidy inhibition (mm) of crude extract and its derived fractions of *Trigonella incise*.

Pathogens	Crude extract	*n*-hexane fraction	Chloroform fraction	Ethyl acetate	*n*-butanol	Aqueous fraction	Antibiotic drug
Pseudomonas aeruginosa	9	6	**11**	6	9	9	14
Escherichia coli	6	5	6	5	**8**	7	11
Salmonella *typhi*	**8**	4	**8**	6	4	4	11
Fusarium oxysporum	9	**10**	7	8	8	6	14
Alterneria alternata	7	8	**10**	7	8	7	13

Table 2. Microbicidy inhibition (mm) of crude extract and its derived fractions of *Nonea edgeworthii*.

Pathogens	Crude extract	*n*-hexane fraction	Chloroform fraction	Ethyl acetate	*n*-butanol	Aqueous fraction	Antibiotic drug
Pseudomonas aeruginosa	7	7	9	5	6	**14**	13
Escherichia coli	7	5	6	**14**	5	7	10
Salmonella typhi	**12**	5	10	9	4	3	15
Fusarium oxysporum	8	7	6	7	**10**	7	15
Alterneria alternata	**11**	7	8	5	8	10	14

Table 3. Anti-oxidative profile of the crude extract and its derived fractions of *Nonea edgeworthii* and *Trigonella incisa* plants.

Sample	*Nonea edgeworthii Trigonella incisa* IC_{50} [µg/mL] [a)] OH^b OH^b
Crude extract	53.27 ± 0.02
n-Hexane	66.35 ± 0.06
$CHCl_3$	62.17 ± 0.03
EtOAc	32.44 ± 0.07
n-BuOH	35.28 ± 0.05
H_2O	90.18 ± 0.02
Trolox[c)]	4.51 ± 0.06

a= Values of OH are expressed as mean ± standard error of triplicate experiments
b= Inhibitory activity of hydroxyl radical generation in 1.0 mM H2O2mMFeSo4
c= Trolox was used as positive control

Table 4. Phytochemical Screening of *Nonea edgeworthii* and *Trigonella incise*.

Plant name	Phytochemicals	Maximum	Moderate	Minimum	Negative
Nonea edgeworthii	Alkaloid		++		
	Saponins			+	
	Flavonoids			+	
	Glycosides				-ive
	Phenol	+++			
	Terpenoids				-ive
	Fatty acid	+++			
Trigonella incise	Alkaloid	+++			
	Saponins		++		
	Flavonoids			+	
	Glycosides				-ive
	Phenol		++		
	Terpenoids				-ive
	Fatty acid		++		

Phytochemical screening: In preliminary photochemical screening alkaloids, phenol and saponins were reported in both plants. Glycosides and terphinoides were found to be absent in both plants (Table 4). While doing our research, the phytochemicals which we have found are familiar in many pharmacological activities. Example of such activity is alkaloids which are generally used as antibacterial, cytotoxic, anti-malarial and anti-cancerous agents (Wirasathien *et al.*, 2006). In the same way in saponins have properties of the insecticidal, fungicidal, antibiotic (Sparg *et al*., 2004). We have found that there is anti-inflammatory, antibacterial, anti-neoplastic, anti-allergic, anti-thrombotic, antioxidants, antiviral and vasodilatory activities in flavonoids (Miller, 1996). Due to pharmacological activities, these compounds are therefore generally found in medicinal plants. Due to high prices of synthetic drugs and because of having a lot of side effects of these synthetic drugs, it is very much necessary for us to produce new useful and safe products for the treatment of different diseases which are caused by human pathogens (Victor *et al*., 2004). The plants for our research have important biological activities that help us to treat various diseases in a traditional manner. Therefore we can take these plants species as an excellent natural source in order to treat several diseases and it might be a powerful targets for the activity guided isolation of its active natural products.

References

Alagumanivasagam, G., R. Pasupathy, A. Kottaimuthu and R. Manavalan. 2012. A review on *In vitro* antioxidant methods. *Inter. J. Pharma. and Chem. Sci*., 1: 662-663.

Ali, S.I. and M. Qaiser. 1993. Flora of Pakistan. Boraginacae. Department of Botany, University of Karachi. 100: 211-216.

Aqil, F., I. Ahmad and Z. Mehmood. 2006. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turk. J. Biol.*, 30: 177-183.

Borris, R.P. 1996. Natural productions research: Perspectives from a major pharmaceutical company. *J. Ethnopharmacol*., 51(1-3): 29-38.

Bruneton, J. 1999. "Pharmacognosie, Phytochimie, Plantes Medicinales" Technique et documentation Lavoisier, Paris.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clinical Microb. Reviews*., 12: 564-582.

Couladis, M., O. Tzakou, E. VerykoKiduo and C. Harwala. 2003. Screening of some Greek aromatic plants for antioxidant activity. *Phytother Research*, 17: 194-195.

Davicino, R., M.A. Mattar, Y.A. Casali, S. Graciela, E. Margarita and B. Micalizzi. 2007. Antifungal activity of plant extracts used in folk medicine in Argentina. *Revista Peruana de Biología*, 14: 247-251.

Dewick, P.M. 2002. *Medicinal Natural Product: A Biosynthetic Approach*. John Wiley West Sussex, UK, 219-226.

Esmaeili, A., B. Rashidi and S. Rezazadah. 2012. Biological activities of various extracts and chemical composition of *Trigonella monantha* C.A. Mey. Sub species *monantha* grown in Iran. *Iranian J. Pharm. Res*., 11: 1127-1136.

Es-Safi, N., S. Khilifa, L. Kerhoas, A. Kollmann, A. El-Abbouyi and P.H. Ducrot. 2005. Antioxidant constituents of the aerial parts of Globulariaalypum growing in Morocco. *J. Nat. Prod*., 68: 1293-1296.

Gilani, S.A., Y. Fujii, Z.K. Shinwari, M. Adnan, A. Kikuchi, K.N. Watanabe. 2010. Phytotoxic studies of medicinal plant species of Pakistan. *Pak. J. Bot*., 42(2): 987-996.

Gohar, Y.M., E.L. Nagghar, M.K. Soliman and K.M. Barakat. 2010. Characterization of marine Burkholderiacepacia antibacterial agent. *J. Nat. Prod*., 3: 86-94.

Harris, C.A., M.J. Renfrew and M.W. Woolridge. 2001. Assessing the risk of pesticide residues to consumers: recent and future developments. *Food Additives and Contamination*, 18: 1124-1129.

Hussain, H., J. Hussain, A. Al-Harrasi and Z.K. Shinwari. 2011. Chemistry of some species genus *Lantana. Pak. J. Bot*., 43 (Special Issue): 51-62.

Kuete, V., J.G. Tangmouo, V.P. Beng, F.N. Ngounou and D. Lontsi. 2006. Antimicrobial activity of the methanolic extract from the stem bark of tridesmoste monomphalocarpoides (Sapotaceae). *J. Ethnophar*., 104: 5-11.

Lopes, M.C. and V.C. Martins. 2008. Fungal plant pathogens in Portugal *Alterneria dauci*. *Revista Iberoamericana de Micología*, 25: 254-256.

Mahesh, B. and S. Satish. 2008. Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World J. Agr. Sci.*, 4: 839-843.

Matin, A., M.A. Khan, M. Ashraf and R.A. Qureshi. 2001. Tradational uses of herbs, shrubs and tree of shogranvaley, Mansehra, Pakistan. *Pak. J. Biol. Sci*., 4: 1101-1107.

McCune, L.M. and T. Johns. 2002. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. *J. Ethnophar*., 82(2-3): 197-205.

Miller, A.L. 1996. Antioxidant flavonoids: Structure, function and clinical usage. 1: 103-111.

Nasir, E. and S I. Ali. 1977. Flora of West Pakistan. Fabaceae. Department of Botany, University of Karachi, 100: 226-230.

Ogunyemi, A.O. 1979. The origin of the herbal cure and its spread. In: *Proceedings of the Conference on African Medicinal Plants*, 20-22.

Preethi, R.M., V.V. Devanathan and M. Loganathan. 2010. Antimicrobial and antioxidant efficacy of some medicinal plants against some food borne pathogens. *Advance in Biol. Res.*, 4: 122-125.

Qasim, M., S. Gulzar, Z. K. Shinwari, I. Aziz and M.A. Khan. 2010. Traditional Ethnobotanical uses of halophytes from hub, Balochistan. *Pak. J. Bot*., 42(3): 1543-1551

Shinwari, Z.K. 2010. Medicinal Plants Research in Pakistan. *Journ. Med. Pl. Res*., 4(3): 161-176.

Shinwari, Z.K., I. Khan, S. Naz and A. Hussain. 2009 Assessment of antibacterial activity of three plants used in Pakistan to cure respiratory diseases. *African Journal of Biotechnology*, 8(24): 7082-7086.

Shinwari, Z.K., M. Rehman, T. Watanabe and Y. Yoshikawa. 2006. Medicinal and Aromatic Plants of Pakistan (A Pictorial Guide). Pp. 492 Kohat University of Science and Technology, Kohat, Pakistan.

Shinwari, Z.K. and M. Qaisar. 2011. Efforts on conservation and sustainable use of medicinal plants of Pakistan. *Pak. J. Bot*., 43 (Special Issue): 5-10.

Siddiqui, BS, M. Hasan, F. Mairaj, I. Mehmood, R. M. Hafizur, A. Hameed, and Z. K. Shinwari. 2014. Two new compounds from the aerial parts of *Bergenia himalaica* Boriss and their anti-hyperglycemic effect in streptozotocin-nicotinamide induced diabetic rats. *Journal of Ethnopharmacology*. 152(3): 561-567.

Sparg, S.G., M.E. Light and J. Staden. 2004. Biological activities and distribution of plants aponins. *J. Ethnophar*., 29: 219-243.

Victor, R., R. Christina, R. Fiona and A. John. 2004. Capturing lay perspectives in a randomized control trial of a health promotion intervention for people with osteoarthritis of the knee. *J. Evaluation In Clinical Practice*, 10: 63-70.

Walter C., Z.K. Shinwari, I. Afzal and R.N. Malik. 2011. Antibacterial activity in herbal products used in Pakistan. *Pak. J. Bot*., 43 (Special Issue): 155-162.

Wirasathien, L., C. Boonarkart, T. Pengsuparp and R. Suttisri. 2006. Biological activities of alkaloids from *Pseuduvariasetosa*. 44: 274-278.

(Received for publication 24 March 2016)