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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *TRICHOSANTHES DIOICA* ROXB. SEEDS

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Abstract

Trichosanthes dioica Roxb (green potato) a genus of family Cucurbitaceae is an ancient ayurvedic medicinal herb and vegetable. Fruit, leaves and tender shoots are used as food system and medicine from time immemorial. It is dioecious plant and mainly distributed throughout North India (Plains), extending to Assam and East Bengal, generally propagated by stem cuttings or root suckers. The current research is focused at the ethno-medicinal plant's phyto-chemical and *in vitro* antioxidant efficiency. The existence of alkaloids, triterpenoids, phytosterols, flavonoids, saponins, amino acids and proteins has been shown by phyto-chemical screening. Antioxidant performance was measured through radical scavenging techniques, using ascorbic acid (standard), 1,1-diphenyl-2-picrylhydrazyl and hydrogen peroxide. The methanol extract was showing antioxidant capacity. Thus, this herb can be used as a potential resource for drug research.

Keywords: *Trichosanthes dioica* Roxb., Phytochemical screening, DPPH and Hydrogen peroxide scavenging activities.

Introduction

Medicinal plants are the backbone of traditional system of medicines, highly valuable and incredible source for treating multiple illnesses (Jawala *et al*., 2009). With a promise of pledge of efficacy and safety, quality herbal plants and products have become priority topic in developing countries and industries. The reliance on synthetics drugs is reduced with the evolution of natural products as novel therapeutic agents for treating various diseases. They are also used ad new entities for the drug discovery. Maximum population of the universe still depends exclusively on herbal plants and products (Kumar and Navaratnam, 2003). These plants having minimum side effect, are easily available and are ecofriendly. Having all these advantages herbal medicines indicates no buzz to be used as valuable source to be used in medicines (Thalluri, 2016).

Many researchers have paid attention towards the Cucurbitaceous family because the fruits, seeds and vegetables are traditionally used in various Ayurveda preparations and confectionary. *Trichosanthes dioica* (pointed gourd), cucurbita species is an important medicinal plant. It is known by various other names like patol, *potala,* sespadula and parwal (Shah and Seth, 2010). As it has good nutritive profile, in Indian market it attains one of the most coveted position during the month of summer and in rainy season. Patola has various chemical constituents like vitamin A and vitamin C, carbohydrates with certain elements like sulphur, magnesium, potassium, chlorine and copper chlorine (Kumar *et al*., 2012). Being an ingredient of sweet, stew, curry, soup it is also eaten fried and as *dolma* with meat stuffing, fish and roe (Saboo *et al*., 2012).

The comestible configuration sections like fruits and vegetables are cooked with meat stuffing in different ways (Singh and Wayne, 1999). The fruits being edible are diuretic in nature having anti-ulcerous effects (Kirtikar and Basu, 1996). Sap of leaves used in acute cases of expansion of liver, spleen and as tonic and febrifuge. Literature from Charaka Samhitha represents its leaves and fruits effective for treating jaundice and alcoholism. Leaves indicate antimicrobial profile. They are also used as antipyretic, cardio tonic, in edema and in alopecia (Khare, 2007). They are employed in treatment of epilepsy, alopecia, skin disease and diabetes mellitus. Many literatures reveal well documented uses of whole plants including leaves and fruits but seeds have not been explored as yet. The present investigation makes an effort to use methanolic extract of the *T. dioica* seeds to explore physicochemical investigation and antioxidant potential of *T. dioica* seeds.

Materials and Methods

Medicine and additives

High-grade testing chemicals and solvents were procured for this study. Hydrogen peroxide (99.4%) was purchased from SDFC Ltd., Mumbai, DPPH from Sigma-Aldrich (Bangalore), and ascorbic acid was procured from Central Drug House (P) Ltd., Baroda.

Plant material

Seeds of *T. dioica* Roxb were acquired from Chandigarh in September month. After selection of healthy seeds, they were identified and authenticated by Department of Botany and Environment, Guru Nanak Dev University, Amritsar, Punjab. A voucher specimen (DUL.Sc. 1811) was deposited in the herbaria of the department for potential tests.

Extract Preparation

Cleaned, dry, powdered (Coarse) seeds were harvested with a mechanical grinder. Powered seeds were exposed to cold maceration with methanol for three consecutive days. The contents were shaken frequently for proper extraction followed by dissipated, condensed in the rotary evaporator at a decreased load. It defatted the condensed filtrate with hexane at least three times with separating funnel. The

collected crude sample was used for several further examinations.

Screening of Phyto-chemical

Crude extort of selected medicinal plant was undergone to qualitative phytochemical screening of phytoconstituents for investigation of alkaloids, saponins, tannins, steroids, anthraquinones, carbohydrates, glycosides and proteins by normal scientific techniques. (Kokate *et al*., 2002; Joseph *et al*., 2013; Sindhu and Arora, 2012). To detect numerous phytoconstituents contained in them, the multiple chemical studies (qualitative) were performed to determine profile of given samples (Table 1).

Table 1: Qualitative chemical tests performed for phytochemical screening.

Phytochemical	Test	Procedure	Observation
Alkaloids	Wagner's test	3-4 ml extract + drops of Wagner's reagent (iodine solution + KI)	Formation of Precipitate (Reddish-brown)
	Dragendorff's test	3-4 ml extract + Dragendorff's reagent drops (Solution of potassium bismuth iodide)	Formation of Precipitate (Orange-brown)
	Mayer's test	5 ml extract + drops of Mayer's reagent (KI solution)	Appearance of colored precipitates (creamy)
	Hager's test	3 ml extract + Some drops of Hager's reagent (picric acid solution)	Presence of coloured precipitates (Yellow)
Carbohydrates	Molisch's test	3 ml extract + α-naphthol in ethanol, 3-4 drops (20%) + 1 ml concentrated sulfuric acid	Presence of a red violet colored ring at the intersection of two layers
	Fehling's test	Extract (1 ml) + Fehling's reagent (2-3 drops) → heating	Brick red colored precipitates
Glycosides	Bontrager's test	Extract (2 ml) + 3 ml benzene → shaking → filtration → filtrate + 5 ml (10% ammonia) → shaking	Creation of a pink ammonia (lower) colour
	Keller-Killani test	Extract (2 ml) + glacial acetic acid (1 ml) + 5% ferric chloride (1 drop) + concentrated H_2SO_4 (1 ml)	Appearance of color (reddish-brown) at intersection of 2 liquids
Flavonoids	Alkaline reagent test	Extract (2 ml) + aqueous sodium hydroxide solution → observe color → add dilute HCl	Yellow colour disappeared on adding dilute HCl
	Shinoda test	3-4 ml extract + magnesium turnings + concentrated HCl from side walls	Magenta color appeared
	Lead acetate test	Extract + lead acetate (2 drops) → stirring	Yellow coloured precipitate formation
Proteins and free amino acids	Millon's test	Extract (2 ml) + Million's reagent (4-5 drops)	Red colored precipitate appeared
	Ninhydrin test	Extract → filtration → filtrate + lead acetate solution → filtration, spotted and ninhydrin spray	Appearance of violet spots
Saponins	Foam test	Extract (2 ml) → diluted with distilled water equivalent to 20 ml→ stirred for 15 minutes	Foam layer formation (1 cm)
	Haemolysis test	Blood (single drop) kept on slide + small amount of the extract	Hemolytic zone appears
Phenolic compounds	Ferric chloride test	Extract (small amount) + 3 ml of distilled water → filtrate + $FeCl_3$ solution (5%)	Appearance of bluish-black colour
	Lead acetate test	Extract (small amount) + lead acetate solution (some drops)	White precipitate formation
Tannins	Gelatin test	2 ml extract + gelatin solution → shaking	White precipitate formation
Phytosterols and triterpenes	Liebermann-Burchard's test	Blend extract and chloroform (3 ml each) + 2 ml acetic anhydride + concentrated H_2SO_4 (2 drops)	Appearance of brick red to blue and finally green colour
	Hesse's reaction	Extract (2 ml) + 4 ml chloroform + 6 ml Conc. H_2SO_4 beside of test tube → shaking	Development of the pink coloured ring which gets diffused in both the layers upon shaking
	Liebermann's test	Combine extract and acetic anhydride (3 ml each) → heating → cooling → H_2SO_4 (few drops)	Blue colour appeared

	Salkowski test	Extract, chloroform and concentrated sulfuric acid (2 ml of each)→ shaking for 5 minutes → allowed to stand for 5 minutes	Red coloured chloroform layer indicates occurrence of sterols and greenish yellow acid layer indicates occurrence of triterpenoids

Antioxidant activity

As the harmful effects of free radicals are increasing on human bodies antioxidants are the center of attraction for many researchers (Molyneux, 2004). Experimentation makes it clear for antioxidant activity assessment (*in vitro*), one standard model of an antioxidant test is not adequate.

Free radical scavenging activities

***In vitro scavenging assay* [2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical)]:** *In vitro* scavenging ability of extracts was assessed qualitatively and quantitatively by TLC autographic and spectrophotometric method, respectively.

***Qualitative estimation of DPPH (free radical) scavenging activity by TLC*:** Silica gel TLC plates analytical (10 cm x 10 cm) were used as preliminary test screening method by using DPPH as spray reagent. The plates were developed using chloroform: methanol: hexane (7:2:1) in order to separate various constituents of the extract. 5μL of each test compound solution (1mg/mL) was applied. The developed plates were air dried plates, DPPH solution (0.2%, MeOH) was sprinkled on to substrate of plates formed with incubation for 10 minutes (room Temp). The involved constituents were visible on purple backdrop as yellow spots. DPPH, purple free standing radical, was limited to yellow hydrazine diphenylpicryl. (Kannan *et al*., 2010; Rajan *et al*., 2011).

***Quantitative estimation of DPPH free radical scavenging action*:** The oxidative scavenging of methanolic extract from *T. dioica* seeds were estimated with minor modifications on the grounds of their secure DPPH free radical scavenging behavior as per the standard method (Subba and Rai *et al*., 2018; Sood *et al*., 2009). DPPH solution (~0.1 mM) was dissolved in ethanol and 1 ml was transferred to 3 ml of test solution formulated with ethanol (25-150 µg/ml). Test of DPPH solution and water deficient sample are used in the testing. Blank provided experiment sample, 60 percent ethanol sample, and DPPH solution without water. The solution was placed for half an hour at ambient temperature (25 ± 2). The absorbance was estimated at 517 nm using UV-visible spectrophotometer (Shimadzu UV-1700 Pharma Spec). All steps have been repeated 3 times. The scavenging potential of DPPH radical has been determined as the following formula:

Capability of scavenging DPPH radical = $(A_c - A_t)/A_c * 100$

Where, A_c and A_t are absorbance's of control and extract specimen, respectively.

Hydrogen peroxide scavenging Study

Hydrogen peroxide generally gets exposed to human beings indirectly through environment with the amount of 0.28 mg $kg^{-1}day^{-1}$, source being crops leaf. Its entry into the human body is through eyes, inhalation and via skin contact. The decomposition products i.e., water and oxygen produce free radical ($OH^{\bullet}$) (hydroxyl radical) causing patho-physiological condition like DNA damage and lipid per-oxidation (Alam *et al*., 2013). The potential of isolate to scavenge hydrogen peroxide as per Ruch *et al*. (1989) system was calculated for *in vitro* analysis. A mixture of H_2O_2 (40 mM) in phosphate buffer was produced and the hydrogen peroxide concentration was estimated spectrophotometrically at 230 nm. Sample (25-150 µg/ml extracts) were applied in purified water (H_2O_2 0.6 ml, 40 mm). After 10 minutes, absorbance was determined at 230 nm against a blank solution using a phosphate buffer without hydrogen peroxide (Dandare *et al*., 2015). The method used to measure scavenging of extracts and regular compounds was:

Percentage inhibition $H_2O_2 = \{(A_o - A_s) / As\} * 100$

Where, A_o and A_s are absorbance's of control and extract.

Results and Discussion

Phytochemical Screening

Foreword phytochemical screening of *T. dioica* seeds indicated the occurrence of diverse plant constituents as presented in Table 2.

Table 2: Phyto-chemical screening of *T. dioica* seed's methanolic extract

Phytoconstituents	Methanolic Extract
Alkaloids, Flavonoids, Saponins, Proteins, Amino acids, Triterpenoids and Phytosterols	+
Carbohydrates, Glycosides, Phenols and Tannins	-

(+) (-) indicates presence and absence of the phytoconstituents.

Qualitative assessment of free radical scavenging study

DPPH method: Qualitative DPPH spray method was done for checking free radical scavenging activity of extort, based on principle that DPPH gets reduced by antioxidants and DPPH purple colour changes to yellow. This is a qualitative antioxidant activity evaluation tool. The color transition from violet-yellow showed that ethanolic extract (seeds) had free radical scavenging potential.

Quantitative assessment of free radical scavenging action

DPPH radical scavenging activity

DPPH molecule is symbolized constant free radical as there is delocalization of electron over the whole molecule which does not allow molecule to dimerize which is the property of many free radicals. It is commonly used to assess molecules aptitude to serve as donors of hydrogen or free radical scavengers. The methodology to DPPH is used in liquid or solid samples and that is not restricted to any particular antioxidant feature, but corresponds to the overall antioxidant potential of the specimen. In DPPH free radical, the odd electron provides a higher maximal absorption (517 nm). The colour changes from violet to yellow when the DPPH radical's molar absorptiveness decreases as unusual DPPH radical electron is combined with hydrogen atom to produce diminished DPPH. Figure 1 demonstrates the composition of DPPH and its inhibition by an antioxidant (Molyneux, 2004).

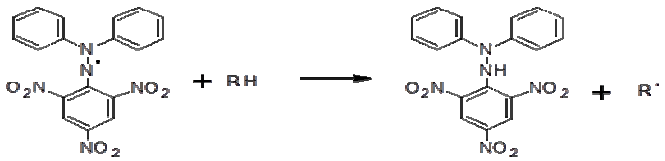

Fig. 1 : Antioxidant reduction of DPPH structure.

With augmenting in concentrate of seeds extract, the antioxidant activity increases proportionally with maximum activity 70.83 % at 150 µg/ml as depicting in Figure 2 and Table 3. It was equivalent to free scavenging outcome of ascorbic acid.

Table 3: DPPH scavenging activity.

Concentration (µg/ml)	Absorbance (test)	Test (% inhibition)	Absorbance (standard)	Standard (% inhibition)
25	0.55±0.020	42.01±2.14	0.47±0.037	51.04±3.89
50	0.47±0.027	50.69±2.73	0.36±0.020	62.5±2.141
75	0.39±0.014	59.71±1.76	0.28±0.016	70.83±1.77
150	0.28±0.011	70.83±0.84	0.18±0.028	81.25±2.99

Values= Mean ± SD; (n=6); *p<0.05.

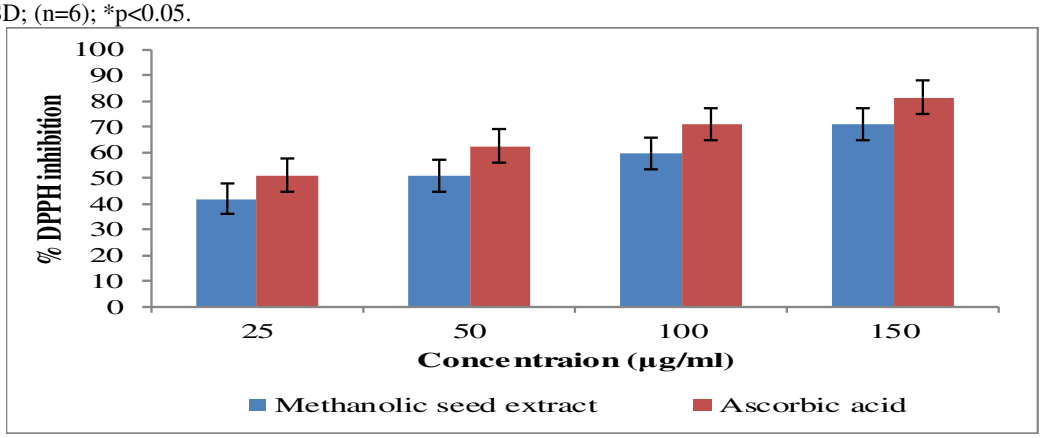

Fig. 2: DPPH scavenging activity. The values are as mean ± SD of n=6 (* p < 0.05)

Hydrogen peroxide radical scavenging activity

H_2O_2 is second experienced climber formed during reduction of molecular oxygen by the gradual electrons. It may also be specifically produced after a reduction of molecular oxygen by two electrons. This can function both as an oxidizing agent and as a reducer. While hydrogen peroxide itself is not very sensitive, cytotoxicity can often be caused by the formation of highly reactive hydroxyl radicals in the cell. So it's very essential to eliminate H_2O_2 from biological systems (Halliwell, 1991). Methanol extract scavenging of H_2O_2 can be due to compounds that can supply electrons to hydrogen peroxide and gets neutralized to water (Halliwell and Gutteridge, 1985). The plant extract's ability to scavenge H_2O_2 may also represent its ability to suppress hydroxyl radical *in vivo* formation (Kellog and Fridovrich, 1975). The seed extract shows radical scavenging behaviour based upon concentration of H_2O_2. Average H_2O_2 scavenging effect was 72.68 % at a conc. of 150 µg/mL, which was equivalent to ascorbic acid scavenging effect. The findings are seen in Table 4 and shown graphically in Figure 3.

Table 4: Scavenging activity of H_2O_2.

Concentration (µg/ml)	Absorbance Mean of test	Test (% inhibition)	Absorbance mean of standard	Standard (% inhibition)
5	0.646±0.019	29.72±1.23	0.612±0.007	33.33±0.84
50	0.555±0.030	36.77±0.85	0.528±0.009	42.48±1.07
75	0.391±0.011	57.36±1.29	0.388±0.033	58.60±3.69
150	0.294±0.002	67.97±2.28	0.250±0.020	72.76±2.28

Values are average ± SD of n = 6; (*p < 0.05)

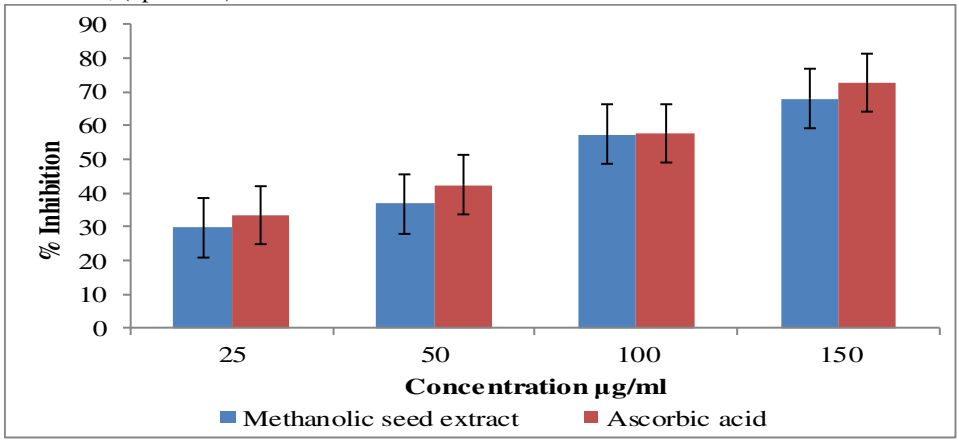

Fig. 3: Hydrogen peroxide scavenging activity. Values= ± SD (n=6); *p<0.05

Conclusion

T. dioica is a popular plant used in Indian system of medicine. Various herbal tests prove it is promising herb, has been in use as folk remedies in different countries for treating various ailments and has wide range of therapeutic actions. Preface phytochemical study of methanol extract of seeds indicated existence of flavonoids, alkaloids, terpenoids and saponins. Antioxidant potential was measured through DPPH and H_2O_2 free radical scavenging action. Outcome confirmed that maximum scavenging effect of extract on DPPH radical was 70.83% which was compared with scavenging effect of ascorbic acid (81.25%) at 150 µg/ml. Scavenging action effect of extract on H_2O_2 radical was 67.97% was analogous to ascorbic acid (72.68%) at 150 µg/ml concentration. Upon separation and detection, various variations of the active constituents can be made and the dynamic effect needs to be further measured. The planning of uniform dose and treatment protocols will play a vital role for humanity in therapeutics.

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Conflict of Interest

Authors declare no conflict of interest.

References

Alam, M.D.; Bristi, N.J. and Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal, 21: 143-152.

Dandare, S.U.; Ezeonwumelu, I.J.; Ezeh, C.P. and Auta, H. (2015). Determination of *in vitro* antioxidant and radical scavenging activities of different extracts of *Allium sativum* (Garlic). IOSR Journal of Pharmacy and Biological Sciences, 9(5): 69-73.

Halliwell, B. (1991). Reactive oxygen species in living systems: source, biochemistry, and role in human disease. American Journal of Medicine, 91: 14-22.

Halliwell, B. and Gutteridge, J.M.C. (1985). Free Radicals in Biology and Medicine. Oxford University Press, Oxford.

Jawala, S.; Gupta, A.K.; Singla, R. and Gupta, V. (2009). General awareness and relative popularity of Allopathic, Ayurvedic and homeopathic system. Journal of Chemical and Pharmaceutical Research, 1(1): 105-112.

Kannan, R.R.R.; Arumugam, R.; Meenakshi, S. and Anantharaman, P. (2010). Thin layer chromatography analysis of antioxidant constituents from seagrasses of Gulf of Mannar Biosphere Reserve, South India. International Journal of Chem. Tech Research, 2(3): 1526-1530.

Kellog, E.W. and Fridovrich, I. (1975). Superoxide, hydrogen peroxide and singlet oxygen in lipid peroxidation by a xanthine oxidase system. Journal of Biological Chemistry, 250(22): 8812-8817.

Khare, C.P. (2007). Encyclopedia of Indian Medicinal Plants. Springer Science and Business media.

Kirtikar, K.R. and Basu, B.D. (1996). Indian Medicinal Plants. Jayyed Press, Allahabad: 1110-1111.

Kokate, C.K.; Purohit, A.P. and Gokhale, S.B. (2002). Pharmacognosy. 13th Ed., Nirali Prakashan, New Delhi.

Joseph, B.S.; Kumbhare, P.H. and Kale, M.C. (2013). Preliminary phytochemical screening of selected medicinal plants. International Research Journal of Science and Engineering, 1(2): 55-62.

Kumar, V. and Navaratnam, V. (2003). Neem (*Azadirachta indica*): prehistory to contemporary medicinal uses to humankind. Asian Pacific Journal of Tropical Medicine, 3(7): 505-514.

Kumar, N.; Singh, S.; Manvi, S. and Gupta, R. (2012). *Trichosanthes dioica* Roxb.: an overview. Pharmacognosy Reviews, 6(11): 61-67.

Molyneux, P. (2004). The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin Journal of Science and Technology, 26(2): 211-219.

Rajan, S.; Mahalakshmi, S.; Deepa, V.M.; Sathya, K.; Shajitha, S. and Thirunalasundari, T. (2011). Antioxidant potential of *Punica granatum* fruit rind extracts. International Journal of Pharmacy and Pharmaceutical Sciences, 3: 82-88.

Ruch, R.J.; Cheng, S.J. and Klaunig, J.E. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 10: 1003-1008.

Saboo, S.S.; Thorat, P.; Tapadiya, G.G. and Khadabadi, S.S. (2012). Distribution and ancient- recent medicinal uses of *Trichosanthes* species. International Journal of Phytopharmacy, 2(4): 91-97.

Shah, B.N. and Seth, A. K. (2010). Pharmacological potential of *Trichosanthes dioica* – an edible plant. Hygeia: Journal for Drugs and Medicines, 2(2): 1-7.

Sindhu, R.K. and Arora, S. (2012). Phytochemical and Pharmacognostical Studies on *Murraya koenigii* (L)spreng. Roots. Drug Invention Today, 4(1): 325-333.

Singh, B.P. and Wayne F. (1999). Whitehead pointed gourd: potential for temperate climates. In: Perspectives on New Crops and New Uses, J. Janick (ed.), ASHS Press, Alexandria: 397-399.

Sood, S.; Arora, B.; Bansal, S.; Muthuraman, A.; Gill, N.S.; Arora, R.; Bali, M. and Sharma, P.D. (2009). Antioxidant, anti-inflammatory and analgesic potential of the *Citrus decumana* L. peel extract. Inflammopharmacology, 17: 267-274.

Subba, A.R. and Rai, S.K. (2018). Phytochemical screening, physico-chemical analysis and antioxidant activity of some ethnomedicinal plants from Sikkim Himalaya. Indian Journal of Natural Products and Resources, 9(3): 235-243.

Thalluri, J. (2016). A review on medicinal plants. Journal of Organometallic Chemistry, 3(2): 157-165.