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Original Article

ESTIMATION OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS OF *THEVETIA PERUVIANA* (PERS.) K. SCHUM

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ABSTRACT

Objective: The aim of this investigation was to estimate antioxidant and antibacterial activity of different crude extracts of *Thevetia peruviana* (Pers.) K. Schum.

Methods: Aqueous and alcoholic crude extracts of different plant parts were obtained and assessed for their antioxidant as well as antibacterial activity. These activities were determined by using standard protocols with some modifications. Aluminium chloride colorimetric method was used to estimate total flavonoid content and total phenolic content was measured by Folin-ciocalteu method.

Results: Free radical scavenging activity was recorded highest in fruits and lowest in root's extracts. The maximum phenolic and flavonoids content was found in fruits (TPC33.59±0.385mg GAE/ g DW, TFC6.98±0.22 mg QE/g DW). Minimum phenol content was found in root's extracts (21.47±0.160mg GAE/ g DW) while leaves possess lowest flavonoid content (4.69±0.17 mg QE/g DW). In case of antibacterial activity, aqueous and ethanol extracts of *Thevetia peruviana* flowers showed maximum antibacterial activity against *Proteus vulgaris* with maximum zone of inhibition with a value of 18.5±0.5 mm and 15.5±1.322 mm respectively. Ethanol extract of *Thevetia peruviana* fruits exhibited maximum antibacterial activity against *Proteus vulgaris* with a value of 13.5±0.5 mm for the zone of inhibition.

Conclusion: This investigation finds that methanol extracts of *Thevetia peruviana* has significant antioxidant activity. These crude extracts can further purify and can be used for development of anti-oxidative pharmaceutical compounds. Aqueous and ethanol extracts of *Thevetia peruviana* fruits and flowers have good antibacterial potential. But care should be taken as the plant has toxic properties also.

Keywords: *Thevetia peruviana*, DPPH, Antioxidant, Antibacterial activity.

INTRODUCTION

Plants have the capability to synthesize a wide variety of chemicals, some of which play an important role in primary metabolic activities while others are part of plant's secondary metabolism and can serve as a source of herbal drug development. The secondary metabolic products like phenolic compound and flavonoids from plants have been reported to have free radical scavenging activity. The presence and distribution of numerous hydroxyl groups in the chemical structure of polyphenols makes them excellent antioxidants. [1, 2] There has been a great deal of interest in the therapeutic potentials of plants, as antioxidants in reducing free radical induced tissue injury.

Reactive oxygen species (ROS) are highly reactive molecules derived from the metabolism of oxygen. ROS, including superoxide radicals, hydroxyl radicals, and hydrogen peroxide. Overproduction of ROS can easily cause oxidative damage to proteins, lipids, lipoproteins and DNA that causes membrane lipid peroxidation, decreased membrane fluidity, and DNA mutations leading to cancer This oxidative damage is a critical factor implicated in several human diseases such as diabetes mellitus, stroke, diabetes, Alzheimer's disease, atherosclerosis, arthritis and neurodegenerative diseases and also in the ageing process. Oxidative stress is ultimately involved in endothelial dysfunction, a condition which is evident in adults suffering from various cardiovascular diseases including thalassemia [3-5].

Thevetia peruviana commonly known as Yellow Oleander is a member of family Apocynaceae. It is commonly found in the tropics and sub-tropics as an ornamental plant, but it is native to Central and South America. It grows to about 10-18 feet high [6]. All parts of the plant contain latex which is toxic. The toxins are cardenolides called thevetin A and B (cereberoside); others include peruvoside, nerrifolin, the vetoxin and ruvo side. They produce gastric and cardio-toxic effects. The plant or its individual parts can be used for the treatment of various disorders in human being such as, diabetes, liver toxicity fungal infection, microbial infection, inflammation, pyrexia and relive pain [7]. Other phyto-constituents from yellow oleander are alkaloids, glycosides, saponins, flavonoids, fixed oils and fats, tannins and phenolic compounds [8]. The plant show effective medicinal properties and reported to have anti- hiv [9], anti- inflammatory [10], antispematogenic [11], antitermite [12], antifungal [13], antioxidant [14], antidiarrhoeal, antimicrobial and cytotoxic activities [15].

The plant possesses many pharmacologically active compounds like alkaloids, glycosides, saponins, flavonoids, tannins and phenolic compounds and has anti- hiv, anti- inflammatory, antispematogenic, antitermite, antifungal, antidiarrhoeal, cytotoxic activities. These useful medicinal activities can form the base for the development of herbal medicines. Hence the plant is selected for this study.

MATERIALS AND METHODS

Plant material

Various plant parts viz, leaves, stem, root, flowers and fruits of *Thevetia peruviana* were collected from University of Rajasthan campus during August- September 2013. Voucher specimen of the plant sample (**RUBL21106)** was deposited in herbarium, Department of Botany, University of Rajasthan, Jaipur. All plant parts were washed with running tap water and shade dried. Then dried plant material powdered using an electric blender.

Chemicals and reagents

2, 2-Diphenyl-1-Picryl Hydrazyl (DPPH), Dimethyl sulphoxide DMSO, Quercetin were procured from Sigma Chemical Co. (St., Louis, USA)., Ascorbic acid, Folin Ciocatteu's reagent, phenol, Gallic acid, anhydrous Sodium carbonate, Aluminum chloride, and Potassium acetate and methanol, Agar, Ethanol. All other chemicals mentioned were obtained from Thermo Fisher Scientific India Pvt. Ltd Powai, Mumbai and Sisco Research Laboratories (SRL) Pvt. Ltd. (Mumbai, India). All chemicals used were of analytical grade. Pure cultures of

all experimental bacteria were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh.

Preparation of plant extracts for antioxidant activity

The dried powder of different plant parts (10 grams each) was soxhlet extracted with methanol for 24 hours. The extract was filtered using whatman filter paper no 1 and filtrates were concentrated to dryness through incubation at room temperature. The extract was preserved for subsequent use in different experiments.

Determination of total phenolic content (TPC)

The total phenolic concentration was measured using the Folin-Ciocalteu method. (16)To the 1 ml of plant extract or standard of different concentrations, 5 ml Folin-Ciocalteu reagent (diluted 10 fold) and 4 ml sodium carbonate solution (0.7 M) was added. Experiment was conducted in triplicates. Calibration curve was prepared using 1 ml methanolic solution of gallic acid (.01-0.4mg/ml) following above mentioned procedure. After incubating for one hour at room temperature, absorbance was measured at 765 nm using spectrophotometer against a reagent blank. Total phenolic content of extracts was expressed as gallic acid equivalents (mg of GAE/ g sample) following formula were used to calculate the total phenolic content.

$T = (C \times V)/M$

T = total phenolic content, mg GAE /gram dry weight of extract

C=concentration of Gallic acid mg/ml

V= volume of plant extract in ml

M= methanolic plant extract weight in grams

Determination of Total Flavonoids Content (TFC)

Aluminium chloride colorimetric method was used to determine the total flavonoid content of plant extracts. (17) To estimate flavonoid content of plant extracts 1 ml of extract was taken and 3 ml methanol added to it. After that 0.2 ml of aluminium chloride (10%), 0.2 ml potassium acetate (1M) and 5.6 ml distilled water were added. Optical densities were measured at 415 nm against a blank. Serial dilution (25, 50,100,200 µg/ml) of methanolic solution of quercetin were used to prepare a standard curve.

The total flavonoid content of extracts was calculated using following formula and were expressed in quercetin equivalent (mg of QE/gdw)

$T=(C \times V)/M$

T= total flavonoid content mg QE /g dry weight of plant extract

C = concentration of quercetin mg/ ml

V= volume of extract in ml

M= plant extract weight in grams

DPPH radical scavenging activity measurement

Free radical scavenging activity of plant extracts was determined by spectrophotometer using the standard protocol for DPPH assay. (18) To1 ml methanolic solution of (0.3) mM DPPH, 1 ml test extracts/standard of different concentrations were added. After incubating for 30 minutes at room temperature in a dark chamber, the absorbance was measured at 517 nm against a control. The percentage scavenging activity was expressed as percentage inhibition of DPPH free radical by the sample and was calculated by using the following expression.

$$\% \text{ inhibition of DPPH} = \frac{\text{Absorbance of control - Absorbance of sample X100}}{\text{Absorbance of control}}$$

Statistical analysis

Results of the experiments are expressed as mean ±S. E. M. All experiments were repeated three times. IC_{50} Values, which is the concentration of the sample that is required to scavenge 50% of DPPH free radicals, were calculated by linear regression curve. Microsoft excel was used for statistical analysis.

Antibacterial assay of *Thevetia peruviana* extracts

Extraction of plant material

50 grams of shade dried and powdered material of flowers and fruits of *Thevetia peruviana* were taken and soxhlet extracted with ethanol and distilled water at 60^0-80^0 for 24-36 hours. The extracts were filtered with whatman filter paper no 1. Each of the extracts dried in vacuo and stored at 4^0C for further experiments.

Test microorganism

Three bacterial strains *Staphylococcus aureus, Bacillus subtilis* (Gram-positive*)* and *Proteus vulgaris,* (Gram-negative) were used in this study. The pure bacterial cultures were maintained on nutrient agar medium. Each bacterial culture was further maintained by sub culturing regularly on the same medium.

Preparation of bacterial suspension

The bacterial suspension was prepared by transferring a loopful of inoculum into normal saline (0.9 %) under aseptic conditions from the stock culture maintained at 4^0C. Density of each microbial suspension was adjusted equal to that of 10^6 cfu/ml (standardized by 0.5McFarland standard) and used as the inoculums for performing agar well diffusion assay.

Screening for antimicrobial activity

The aqueous and ethanol extracts of yellow oleander fruits and flowers were used for evaluation of antimicrobial activity by the agar well diffusion method. 50 µl of inoculum of each test organism was spread onto the agar plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells of 6 mm were made with a sterile borer in the inoculated agar plates. The dried extracts were reconstituted in dimethyl sulphoxide (DMSO) for the bioassay analysis. A 100 µl volume of each extract was propelled directly into the wells (in triplicates) of the inoculated agar plates for each test organism. The plates were allowed to stand for 1hr for diffusion of the extract into the agar and incubated at 37^0C for 24h. Sterile DMSO served as the negative control and streptomycin for bacteria served as the positive control. The antimicrobial activity, indicated by an inhibition zone surrounding the well containing the extract, was recorded if the zone of inhibition was greater than 8 mm.

RESULTS AND DISCUSSIONS

Total Phenolic Content (TPC)

Specific phenolic compounds show health promoting properties with antioxidant activities towards cancer, cardiovascular and neurodegenerative diseases or for use in anti aging.(19) Maximum content of phenol compounds (33.59±0.38 mg GAE/gm dry weight)was found in fruits and least concentration was found in the roots (21.47±0.16 mg GAE/gm dry weight) of *Thevetia peruviana*. Folin-Ciocalteu method was used to measure TPC value in different plant extracts. Results are expressed as gallic acid equivalents/gram dry weight of the plant extract.

Total Flavonoid Content (TFC)

Flavonoids are excellent antioxidant compounds which reduce the level of reactive oxygen species by their hydroxyl groups. Maximum flavonoid content was found in fruits (6.98±0.22 mg QE/gm Dry Weight) and lowest content was found in leaves (4.69±0.17 mg QE/gm Dry Weight) of yellow oleander. TFC values of different extracts of the plant were determined by Aluminium chloride colorimetric method.

The results of all experiments are given in table no: 1 and a comparison of total phenol and flavonoid content of crude extract of *Thevetia peruviana* is given in fig. 1.

DPPH radical scavenging activity

DPPH radical assay is widely used to estimate free radical scavenging activity of different compounds. DPPH is known to obstruct labile hydrogen. Scavenging of free radicals is one of the major anti-oxidation mechanisms to inhibit chain reaction of lipid peroxidation. DPPH radical scavenging activity is related to the

inhibition of lipid peroxidation. This investigation involves the use of different crude extracts of plant's parts to find out radical scavenging activity.

Results of the experiments clearly show that roots have minimum antioxidant activity with maximum IC_{50} value of 170.18±0.77µg/ml while fruits show maximum antioxidant activity with minimum IC_{50} value of 107.37±0.35µg/ml. Root, flower, leaves, stem and fruits of *Thevetia peruviana* show anti-oxidative potential in an increasing order. These extracts can act as free radical scavengers by preventing and repairing damages caused by reactive oxygen species such as superoxide anion, hydroxyl radical and hydrogen peroxide. This antioxidant activity forms a strong base for development of useful bio-inspired medicines to reduce free radical damage to cell [20].

Table 1: Total phenol and flavonoids content in different crude extracts of *Thevetia peruviana* (Pers.) K. Schum.

Plant Parts	Total phenol content mg GAE/g dry weight of extract	Total flavonoid content mg QE/ g dry weight of extract
Leaf	25.57 ± 0.09	4.69±0.17
Stem	31.26±0.15	6.47±0.34
Root	21.47±0.16	5.21±0.23
Fruit	33.59±0.38	6.98±0.22
Flower	32.79±0.32	5.49±0.39

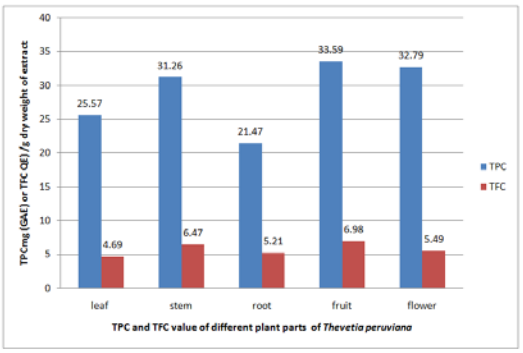

Fig. 1: Total phenol and flavonoids content in *Thevetia peruviana*

IC_{50}values of crude extracts of different plant parts are shown in table no. 2 and fig. 2 to fig. 6 are showing their DPPH radical scavenging activity.

Table 2: IC_{50} Values of different plant parts of *Thevetia peruviana* (Pers.) K. Schum.

Plant Parts	IC_{50} values (µg/ml)
Root	170.18±0.77
Stem	121.74±0.19
Fruit	107.37±0.35
Flower	164.15±0.11
Leaf	151.02±0.24
Ascorbic acid	16.33 ± 0.32

Antibacterial activity

The antibacterial activity of aqueous and ethanol extracts of fruits and flowers of *Thevetia peruviana* was assayed *In vitro* by an agar disc diffusion method against three different bacterial strains namely *Staphylococcus aureus, Bacillus subtilis* (Gram positive),and *Proteus vulgaris* (Gram negative). The aqueous extracts of yellow oleander flowers had greater antibacterial activity when compared to alcoholic extract.

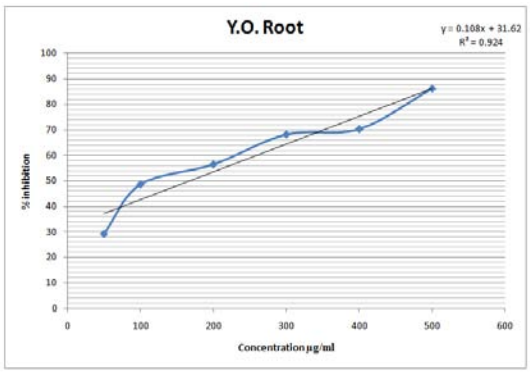

Fig. 2: DPPH Radical scavenging assay of *Thevetia peruviana* root

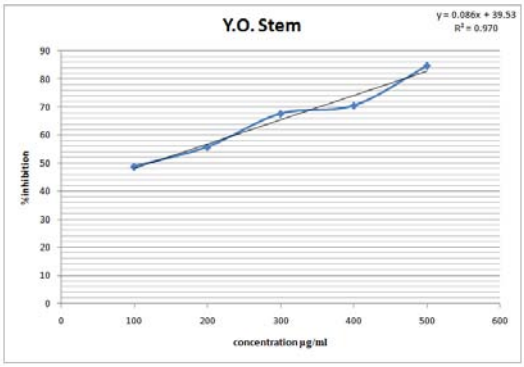

Fig. 3: DPPH Radical scavenging assay of *Thevetia peruviana* stem

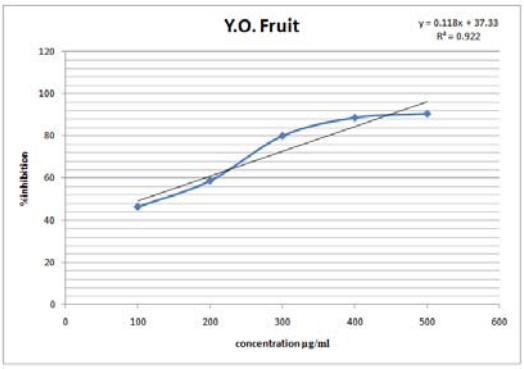

Fig. 4: DPPH Radical scavenging assay of *Thevetia peruviana* fruit

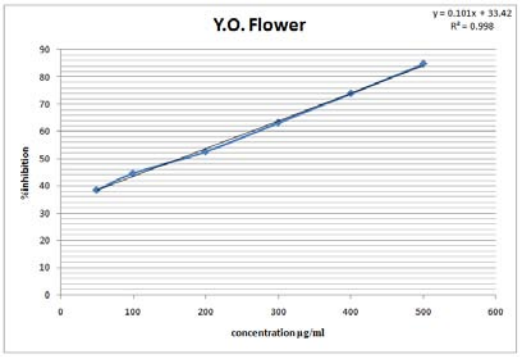

Fig. 5: DPPH Radical scavenging assay of *Thevetia peruviana* flower

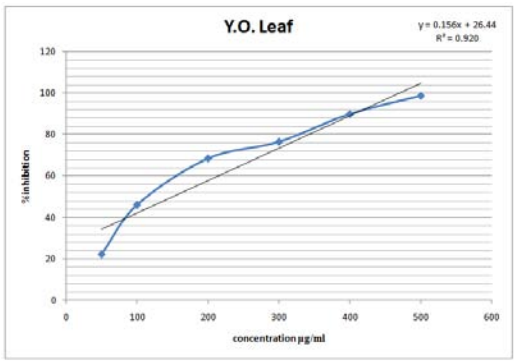

Fig. 6: DPPH Radical scavenging assay of *Thevetia peruviana* leaves

Floral extracts, namely aqueous and ethanol, showed maximum antibacterial activity against *Proteus vulgaris* with maximum zone of inhibition with a value of 18.5±0.5 mm and 15.5±1.322 mm respectively. In case of fruit extracts of *Thevetia peruviana* ethanol extracts exhibited maximum antibacterial activity against *Proteus vulgaris* with a value of 13.5±0.5 mm for zone of inhibition.

When ethanol and aqueous extracts of both plant parts were compared, aqueous extracts of *Thevetia peruviana* flowers exhibited maximum antibacterial activity. The results of these experiments suggest that these extracts have good potential for developing bio-inspired antibacterial drugs.

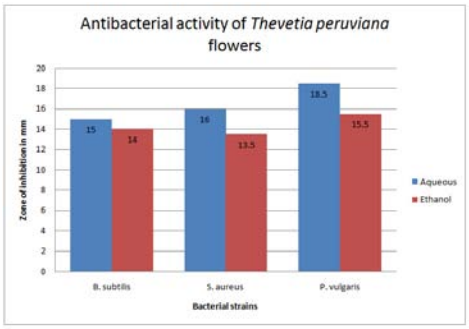

Fig.7: Antibacterial activity of *Thevetia peruviana* flowers

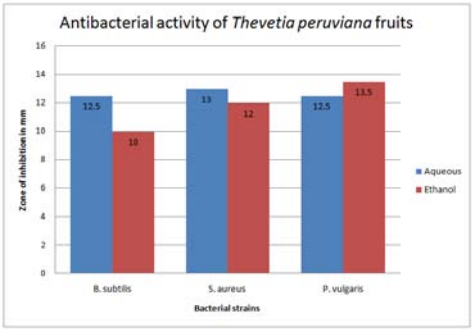

Fig. 8: Antibacterial activity of *Thevetia peruviana* fruits

Table 3: Antibacterial activity of *Thevetia peruviana* flowers and fruits extracts

Plant parts	Solvent	Diameter of zone of inhibition (10mg/ml)		
		B. subtilis	***S. aureus***	***P. vulgaris***
Flowers	Water	15±1	16±1.732	18.5±0.5
	Ethanol	14±1.732	13.5±1.802	15.5±1.322
Fruits	Water	12.5±0.5	13±1	12.5±1.5
	Ethanol	10±1	12±1	13.5±0.5
Standard	Streptomycin	21	18	20

CONCLUSION

The findings of this study are the presence of significant antioxidant and antibacterial activity in aqueous and alcoholic extracts of different parts of *Thevetia peruviana*. Total phenolic and flavonoid contents that plant possesses are in the good amount. This can be concluded that there is sufficient amount of compounds having activity to scavenge reactive oxygen species. The plant extracts show promising free radical scavenging activity and comparable activity against gram positive and gram negative bacteria. There is a good scope to develop natural drugs to reduce oxidative stress and to fight against bacterial pathogens. But care should be taken as the plant has toxic properties also. Further investigation is recommended for isolation and development of a non poisonous antioxidant and antibacterial pharmaceutical compounds from crude extracts of various plant parts.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Zibbu G, Batra A. *In vitro* and *in vivo* determination of phenolic contents and antioxidant activity of desert plants of apocynaceae family. Asian J Pharm Clin Res 2012;5(1):75-83.
2. Gawron-gzella A, Dudek-makuch M, Matławska I. DPPH radical scavenging activity and phenolic compound content in different leaf extracts from selected Blackberry species. Acta Biol Cracov Ser Bot 2012;54(2):32-8.
3. Patel VR, Patel PR, Kajal SS. Antioxidant activity of some selected medicinal plants in western region of india. Adv Biol Res 2010;4(1):23-6.
4. Aqil F, Ahmad I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used indian medicinal plants. Turk J Biol 2006;30:177-83.
5. Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. Afr J Biotechnol 2008;7(18):3188-92.
6. Usman LA, Oluwaniyi OO, Ibiyemi SA, Muhammad NO, Ameen OM. The potential of Oleander (*Thevetia peruviana*) in African agricultural and industrial development: a case study of Nigeria. J Appl Biosci 2009;24:1477-87.
7. Kumar A, Singh S, Mahour K, Vihan VS, Gururaj K. Phytochemical analysis of some indigenous plants potent against ectoparasite. Asian J Exp Biol Sci 2011;2(3):506-9.
8. Rajbhar N, Kumar A. Pharmacological importance of *thevetia peruviana.* Int J Pharma Chem Sci 2014;3(1):260-3.
9. Tewtrakul S, Nakamura N, Hattori M, Fujiwara T, Supavita T. Flavanone and flavonol glycosides from the leaves of *thevetia peruviana* and their hiv-1 reverse transcriptase and hiv-1 integrase inhibitory activities. Chem Pharm Bull 2002;50(5):630-5.
10. Thilagavathi R, Kavitha HP, Venkatraman BR. Isolation, Characterization and anti-inflammatory property of *thevetia Peruviana*. E-J Chem 2010;7(4):1584-90.
11. Gupta R, Kachhawa JB, Gupta RS, Sharma AK, Sharma MC, Dobhal MP. Phytochemical evaluation and antispermatogenic activity of *Thevetia peruviana* methanol extract in male albino rats. Hum Fertil 2011;14(1):53-9.

12. Amal TT, Kwadwo TS, Paul BP, Hawkins EJ. The termite controlling capabilities of extracts of *Thevetia peruviana* (Pers.) K. Schum. In Ghana. ICJEST 2014;1(1):1-8.
13. Ambang Z, Ngoh Dooh JP, Essono G, Bekolo N, Chewachong G, Asseng CC. Effect of *Thevetia peruviana* seeds extract on *In vitro* growth of four strains of *Phytophthora megakarya.* POJ 2010;3(3):70-6.
14. Nesy EA, Jolly C, Mathew L. Assessment of *In vitro* antimicrobial and antioxidant potential of *thevetia neriifolia*, juss. flower morphoforms. J Pharm Biol Sci 2014;9(2):11-6.
15. Hassan MM, Saha AK, Khan SA, Islam A, Mahabub-Uz-Zaman M, Ahmed SSU. Studies on the antidiarrhoeal, antimicrobial and cytotoxic activities of ethanol-extracted leaves of yellow oleander (*Thevetia peruviana).* Open Veter J 2011;1:28-31.
16. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem 2001;73:73-84.
17. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002;10:178-82.
18. Blois MS. Antioxidant determination by use of Stable free radicals. Nat 1958;29:1199-200.
19. Boudet AM. Evolution and current status of research in phenolic compounds. Phytochem 2007;68(22-24):2722-35.
20. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, Bekhradnia AR. Antioxidant and free radical scavenging activity of *H. officinalis* L. Pak J Pharm Sci 2010;23(1):29-34.