ANTIOXIDANT PROPERTIES AND PHYTOCHEMICAL PRINCIPLES OF SOME MEDICINAL PLANTS: *ADENIUM OBESUM* (FORSSK.) ROEM. & SCHULT., *IXORA COCCINEA LINN.* AND *AEGLE MARMELOS* LINN.

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خلاصہ

Ademium obesum, Ixora coccinea اور Aelgle marmelos پودوں کے میتھانول استخراج، اس کے اجزاء اور ماتحت اجزاء کی DPPH طریقہ کار پر بیونائلیٹڈ ھائیڈروآکسی ٹولین (BHT) اور اسکاربک تراشہ (ascorbic acid) کو معیار بناتے ہوئے ضد تکسیدی (Antioxidant) سرگرمی معلوم کی گئی۔ Ixora coccinea نے سب سے زیادہ پھر Ademium obesum اور Aelgle marmelos نے سرگرمی ظاہر کی۔ Ixora coccinea کے پتوں کے استخراج نے تمام دیگر استخراجوں کے مقابلے میں سب سے زیادہ گرمی IC50 = 0.00315 ± 0.0001 mg/ml ظاہر کی۔ سرگرمی ظاہر کرنے والے اجزاء کی تخلیص کے نتیجے میں چھبیس (1-26) مرکبات حاصل ہوئے حاصل شدہ محاصلات کی بھی تکسیدی (Antioxidant) سرگرمی معلوم کی گئی جن میں سے خالص مرکب-5-0-caffeoly quinic (19) (Ixora coccinea کے پھولوں کے استخراج کے جز سے حاصل کیا گیا) نے سب سے اچھی (Antioxidant) سرگرمی (IC50 =0.0467 ± 0.0018 mg/ml) اسکاربک ترشہ (IC50 = 0.0491 ± 0.0009) اور 3HT (IC50 = 0.0407 ± 0.003) کو معیار (standard) مانتے ہوئے ظاہر کی۔

Abstract

The methanolic extracts, fractions and sub-fractions of *Adenium obesum* (Forssk.) Roem. & Schult., *Ixora coccinea Linn.* and *Aegle marmelos* Linn. were screened for their free radical scavenging ability by using ascorbic acid and butylated hydroxytoulene (BHT) as standard antioxidants and evaluated through DPPH free radical scavenging assay. *I. coccinea* exhibited strongest inhibition, followed by *A. obseum,* and *A. marmelos*. The methanolic extracts of *I. coccinea* leaves proved to be the most potent antioxidant among these extracts with IC_{50} 0.00315 ± 0.0001 mg/mL. Upon the purification of the active fractions twenty-six (**1-26**) pure chemical constituents were isolated and examined for their antioxidant inhibition ability, out of which 5-*O*-caffeoyl quinic acid (**19**) isolated from the active fraction of *I. coccinea* flower found to be potent antioxidant with IC_{50} value 0.0467 ± 0.0018 mg/mL as compared to standard ascorbic acid (0.0491 ± 0.0009) and BHT (0.0407 ± 0.003).

Introduction

In the human body free radicals are formed by the endogenic metabolic reactions, these are the unstable species which are responsible for oxidative damage to biomolecules. They can trigger various diseases such as cancer, aging, neurological disorders, arthritis, cirrhosis & atherosclerosis (Halliwell and Gutteridge, 1984; Maxwell, 1995; Badarinath *et al.*, 2010). Antioxidants are used to refrain the harmful effects of free radicals which reduces the risk of these disorders (Rice-Evans *et al.*, 1996; Chakraborty *et al.*, 2009). Almost all organisms have some mechanism to cope up with the damage caused by free radicals with the help of enzymes such as super-oxide dismutase,

glutathione peroxidase, catalase and antioxidant compounds viz. ascorbic acid, phenolic acids, polyphenols, tocopherol, flavonoids as well as antioxidant supplements also provides protection against the hazardous effects of free radicals (Prior and Cao, 1999). Nowadays the use of natural antioxidants is preferable. Numerous medicinally active plants have been shown such efficacy through the traditional approaches of psychoneuropharmacology (Koslow *et al.*, 1995). For this purpose, we selected three plants for their famous medicinal potential and traditional uses.

Adenium obesum, usually known as desert rose, belongs to the family Apocynaceae (Omino and Kokwaro, 1993) found all over Africa and Southern Africa (Magassouba *et al.*, 2007) and reported as a rich source of cardiac glycosides, pregnanes, triterpenes, steroids, flavonoids and carbohydrates (Versiani *et al.*, 2014; Ahmed *et al.*, 2017). Literature review revealed that *A. obesum* possesses antibacterial, antitumor, antiviral, immunomodulatory activity and toxicity whereas depth antioxidant activity has explored on the roots of *A. obesum* (Al-Ghudani and Hossain, 2015).

Ixora coccinea Linn., a traditional medicinal plant which is also known as Jungle Geranium and Flame of wood. It is distributed in the tropical and sub-tropical regions of world (Baliga *et al.*, 2012). It has been reported that *I. coccinea* possess extensive biological activities such as hepatoprotective, antimicrobial, antioxidant, antinociceptive, anti-mitotic, anti-inflammatory along with cardiovascular activity (Elumalai *et al.*, 2012; Rahman *et al.*, 2012). It is a rich source of various bio-active class of compounds such as alkaloids, flavonoids, tannins, steroids and terpenoids (Donth *et al.*, 2015; Versiani *et al.*, 2012; Ikram *et al.*, 2016; Ikram *et al.*, 2013).

Aegle marmelos Linn. Correa, known as beal fruit tree (Chakthong *et al.,* 2012). It is a sub-tropical species widely found throughout India, Ceylon, Burma, Bangladesh, Bhutan, Malaysia and Pakistan (Dhankar *et al.*, 2011). Medicinally, every part of the tree has great importance and every part is used in indian medicinal system (Brijesh *et al.*, 2009). As a whole plant, it possesses antiviral, antidiarrheal, antimicrobial, radio protective, anticancer, chemo-preventive, antiulcer, antipyretic, antioxidant, antigenotoxic, diuretic, antimalarial and anti-inflammatory properties, which helps to play a key role in prevention and treatment of several diseases (Arunachalam *et al.*, 2014). Flavonoids, alkaloids, steroids, terpenoids, saponins, tannins, coumarin derivatives, amino acid and carbohydrates have been reported from this plant (Charoensiddhi and Anprung, 2008). Due to their large number of medicinal uses with the presence of valuable constituents need to explore their antioxidant potential. Keeping this in view, the present study has been made to assess the relative antioxidant potential of *A. obesum, I. coccinea* and *A. marmelos,* which are traditionally used in various medications.

Materials and Methods

Plant materials

To isolate the active chemical constituent from *A. obesum* and *I. coccinea* different parts (leaves, twigs, pods, flowers and roots) of these plants were collected from the garden of University of Karachi. The plant was authenticed by Dr. Rubeena Dawar and Dr. Sahar Ansari respectively at the Department of Botany, University of Karachi and vouchers specimen number KUH 68671 and KUH 68501, respectively were deposited in the herbarium of the same department.

Wet and uncrushed fruits (475 g) of *A. marmelos* were purchased from the local market of Karachi. The plant material was identified by Dr. Bina Naqvi; Taxonomist at PCSIR Laboratory complex, Karachi, Pakistan. The specimen voucher (Pharm-AM-0012/2013) was deposited in the herbarium of Faculty of Pharmacy, Federal Urdu University, Gulshan-e-Iqbal campus, Karachi, Pakistan.

Chemicals

Hexane, chloroform, ethyl acetate, butanol, methanol, dimethylsulfoxide (DMSO) and ascorbic acid ($C_6H_8O_6$) were purchased from Merck (Dramstadt, Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich and butylated hydroxytoulene (BHT) was purchased from BDH Laboratory Supplies, Poole, BH15ITD, England. Vacuum liquid chromatography (VLC) was performed on silica gel $60GF_{254}$ and flash column chromatography (FCC) on silica gel 60 (E. Merck 1.09385) (Model Aldrich), while size exclusion chromatography was acheived by using Sephadex LH-20 (Amershem Bioscience), pre-swollen in the specified solvent before loading on to the column.

Extraction, fractionation and purification

Powder of Flower (500 g) of *A. obesum* was extracted in methanol and the crude extract (AOFM, 100 g) was partitioned between aqueous, ethyl acetate (AOFMEA, 20 g), butanol (AOFMBut, 5 g), methanol (AOFMM, 10 g),

and 70% methanol (AOFM-70, 25 g). The EA-phase (AOFMEA) was dried with sodium sulfate (anhydrous), charcoaled and evaporated invacuo to give a thick residue which was subjected to normal pressure column chromatography on gradient system by using hexane, chloroform and methanol and afforded the five pure compounds obeside D (**1**, 10 mg), obebioside D (**2**, 5 mg), Δ^{16}-digitoxigenin-*β*-D-glucopyranosyle-*β*-D-digitalose (**3,** 19 mg), *β*-sitosterol (**4**, 3 mg) and stigmasterol (**5,** 10 mg) (Ahmed *et al.*, 2017). Fruits (wet pods, 481 g), fruit (dry pods, 20 g), leaves (112 g) and roots (3 Kg) of *A. obesum*, were extracted in methanol and got residue AOWPM, AODPM, AOLM and AORM, respectively. The methanolic extract of wet pods (AOWPM) was partitioned between aqueous and ethyl acetate phase. The anhydrous ethyl acetate phase was charcoaled and evaporated invacuo to give a residue. The residue was further fractioned by using petroleum ether (100%), petroleum ether: ethyl acetate (1:1), ethyl acetate (100%) and methanol (100%). The ethyl acetate soluble sub-fraction of ethyl acetate was subjected to normal pressure column chromatography on gradient system as mentioned above and obtained two pure chemical constituents neritaloside (**6,** 4 mg) and ursolic acid (**7**, 5 mg) (Ahmed *et al.,* 2017). Petroleum ether: ethyl acetate soluble sub-fraction of ethyl acetate fraction was purified by vacuum liquid chromatography (VLC) followed by gel filtration column (Sephadex, LH-20) chromatography of combined VLC fractions 31 to 40 gave two pure compounds dihydroifflanoic acid (**8**, 10 mg) and (**7**, 21 mg) (Ahmed *et al.*, 2017) while normal pressure column chromatography of combined VLC fraction nos. 51 to 100 (500 mg) on gradient solvent system using hexane, ethyl acetate and methanol by increasing 5% polarity afforded a pure compound honghelin (**9**) (Ahmed *et al*., 2017).
In another work 3 Kg of the fruits (pods) were soaked in MeOH (5 L) at room temperature for a month and get the extract (150 g) which was partitioned by using water and ethyl acetate. The ethyl acetate phase (27 g) was further fractionated by using hexane and methanol. The later fraction (21.5 g) was subjected to flash column chromatography followed by normal pressure column chromatography and reversed phase High Pressure Liquid Chromatography (HPLC, Dionex 300, C18 column, 250mm × 25mm, isocratic system, flow rate 0.2 ml/min, 100 % acetonitrile, 254 nm) afforded eight pure compounds obeside B (**10**, 5 mg), obeside C (**11**, 7 mg), betulinic acid (**12**, 3 mg), oleanolic acid (**13**, 5 mg), **7** (25 mg), **8** (30 mg), **6** (11 mg) and gitoxigenin-*β*-D-glucopyranosyle-(1→4)-*β*-D-digitalose (**14,** 5 mg) (Ahmed *et al*., 2017)(Fig 1).

The fresh leaves (2 Kg) and twigs (2.2 Kg) of *I. coccinea* were extracted with methanol which gave thick residues IXLM (213 g) and IXTM (225 g) on evaporation. IXLM was partitioned into six fractions by using non-polar to polar solvents petroleum ether, dichloromethane, dichloromethane: ethyl acetate (1:1), ethyl acetate, ethyl acetate: methanol (1:1) and methanol which afforded $IXLM_1$, $IXLM_2$, $IXLM_3$, $IXLM_4$, $IXLM_5$ and $IXLM_6$ fractions respectively along with insoluble fraction. In fraction $IXLM_4$ some white crystals are settled down which were separated and identified as D-mannitol (**15**, 30 mg) (Versiani *et al.,* 2012). $IXLM_1$ was purified through preparative thin layer chromatography (PTLC) (silica gel, hexane: ethyl acetate, 6.5: 3.5) and obtained a pure compound 17*β*-dammara-12, 20-diene-3*β*-ol (Ixorene **16**, 5 mg) (Ikram *et al.*, 2013). Fresh flowers of *I. coccinea* (746 g) were extracted in methanol and obtained a thick residue IXFM which was partitioned by using water (IXFMAq) and ethyl acetate (IXFMEA). EA-phase (IXFMEA) was fractionated into petroleum ether, petroleum ether: ethyl acetate (1:1), ethyl acetate and methanol, afforded four fractions IXF_1 (5.4493 g), IXF_2 (200 mg), IXF_3 (2.62 g), IXF_4 (5.14 g) together with insoluble material respectively, in which IXF_1 form three layers which were separated into IXF_1BA, IXF_1BB and IXF_1BC. Some solid material settled down in fraction IXF_4 and obtained as IXF_{4b} fraction. To purify the fraction IXF_2, performed vacuum liquid chromatography (VLC) followed by preparative thin layer chromatography (PTLC) two pure compounds stigmast-5-en-3-O-*β*-D-glucoside (**17**, 6 mg) and stigmast-5-en-3-*O*-*β*-D-tetraacetoxyglucoside (**18**, 10 mg) obtained (Versiani *et al.*, 2012). Fraction IXF_3 and IXF_4 were subjected to normal pressure column chromatography which afforded 5-*O*-caffeoylquinic acid (**19**, 30 mg) and 19,21-epoxy-tirucall-7-en-3-ol (Ixoroid **20**, 10 mg) (Versiani *et al.*, 2012) respectively, while upon the purification of fraction IXF_1BC (3 g) obtained 5*α*-pregn-9(11)-17(20)-diene (**21**, 10 mg), 17-*β*-Dammara-12,20-diene-3-*β*-isovelarate (Ixorene isovalerate **22**, 10 mg), 17-*β*-dammara-12,20-diene-3-*β*-30,80-dimethyloctanoate (**23**, 10 mg), 3-acetoxyursolic acid (**24**, 5 mg) and 3-*β*-hydroxy-18-*β*-urs-12ene-29-oic acid (Ixoroid acid **25**, 15 mg) (Ikram *et al.*, 2016)(Fig 2).

A. marmelos fruits were soaked into 70% methanol, filtered and evaporated in vacuuo to give extract (AMFM, 250 g). The extract (AMFM) was fractionated from non-polar to polar fractions by using hexane, ethyl acetate, butanol and methanol which gave the soluble fractions AMFMH, AMFMEA, AMFMBut and AMFMM respectively. For further sub-fractionation of ethyl acetate fraction, AMFMEAHEA, AMFMEAEA and AMFMEAM were obtained by using hexane: ethyl acetate (1:1), ethyl acetate and methanol respectively. AMFMButHEA, AMFMButEA, AMFMButM and AMFMButMI (white crystals) were the hexane: ethyl acetate (1:1), ethyl acetate, methanol sub-fractions and insoluble fractions of butanol soluble fraction respectively. On evaporation of the solvents of sub-fraction AMFMButHEA a solid material was obtained which was re-crystallized and got a pure off-white shiny crystalline compound identified as a well-known constituent marmelosin (**26**, 10 mg)

(Dhankar *et al.*, 2011). AMFMMH (hexane), AMFMMHEA (hexane: ethyl acetate; 1:1), AMFMMEA (ethylacetate) and AMFMMM (methanol) were the sub-fractions of methanolic soluble fraction of main extract of *A. marmelos* fruits.

All of these extracts, fractions, sub-fractions and pure isolated compounds of *A. obesum*, *I. coccinea* and *A. marmelos* were evaluated for their antioxidant activity (Table 1).

1.2.2. Determination of radical-scavenging activity

DPPH [1, 1-diphenyl-2-picrylhydrazyl] is a free radical which is stable in nature. It shows the absorbance at 520 nm and has the violet color which then become colorless after the action of antioxidants. The free radical scavenging potential was carried out by using DPPH assay according to standard protocol (Badrinath *et al.*, 2010; Ikram *et al.*, 2016).

Statistical analysis

Data were expressed as mean ± standard deviations (SD) of three replicated determinations and IC_{50} values of all experiments were calculated by using EZ-Fit Enzyme Kinetic software. All statistical analysis was carried out by using SPSS version 20.0

Results and Discussion

The antioxidant activity of extracts of different parts (fruits, flowers, leaves, roots and pods), their fractions, sub-fractions and pure isolated compounds of the selected medicinal plants were summarized in Table 1.

In the present study, the methanol extracts of *A. obesum,* AODPM (dried pods), AOWPM (wet pods), AOLM and AOFM were showed potent inhibition with IC_{50} values 0.2242 ± 0.0020, 0.0951 ± 0.003, 0.1198 ± 0.0107, and 0.0395 ± 0.0023 mg/mL respectively and significant difference between the inhibitory effects at different concentrations was observed ($P < 0.05$). Fractions AOFMM-70, AOFMM and AOFMEA were also active against DPPH radical with IC_{50} values 0.0584 ± 0.0015, 0.2752 ± 0.0001, and 0.1502 ± 0.0029 mg/mL (Table 1; Fig. 3). However, the roots extract (AORM) was found to be inactive at the concentration 0.5 mg/mL. All the pure compounds isolated from flowers and pods of *A. obesum* found to be inactive at the concentration of 0.5 mg/mL as compare with standards drugs ascorbic acid and BHT (Table 1; Fig. 3) as the pure isolated compounds were belongs to the cardiac glycosides, terpenoids and steroids. The methanol extract of *I. coccinea* twigs (IXTM) and leaves (IXLM) were exhibited the potent antioxidant potential with IC_{50} values 0.0035 ± 0.0003 and 0.00315 ± 0.0001 mg/mL respectively as compare with standard drug ascorbic acid (Table 1; Fig. 3) and significant difference between the inhibitory effects at different concentrations was observed ($P < 0.05$). Among the leaves fractions $IXLM_1$, $IXLM_2$, $IXLM_4$, $IXLM_5$ and $IXLM_6$ have ability of inhibition with IC_{50} values 0.0023± 0.0001, 0.1881± 0.0007, 0.0016± 0.0004, 0.0267 ± 0.0016 and 0.0130 ± 0.0057 mg/mL respectively except $IXLM_3$ fraction. Two pure compounds isolated from the leaves of *I. coccinea* D-mannitol (**15**) and ixorene (**16**) were examined for antioxidant potential but both found to be inactive at the concentration of 0.5 mg/mL as compared to the standard drugs ascorbic acid and BHT. Ixorene belongs to the class of triterpenoids and was reported as new natural product (Ikram et al. 2013. The methanol extract of *I. coccinea* flower (IXFM) also showed antioxidant activity with IC_{50} value at 0.0459 ± 0.0027 and significant difference between the inhibitory effects at different concentrations was observed ($P < 0.05$). The fractions IXFMAq and IXFMEA, sub-fractions IXF_1, IXF_2, IXF_3, IXF_4, IXF_{4b}, IXF_1BA, IXF_1BB, IXF_1BC were found to be more active with IC_{50} values 0.0491± 0.0010, 0.0048 ± 0.0004, 0.0061± 0.0001, 0.0032 ± 0.0001, 0.0015±0.0003, 0.0023± 0.0004, 0.0302 ± 0.0014, 0.0652± 0.0008, 0.0615± 0.0001 and 0.0478 ±0.0001 mg/mL respectively. Compounds (**17-25**) isolated from the flower of *I. coccinea* were belongs to steroids, terpenoids and polyphenols, in which compounds **20**, **22**, **23** and **25** were reported as new natural products (Versiani et al. 2012; Ikram et al. 2016). All these isolated compounds were examined for their antioxidant potential out of which only 5-*O*-caffeoyl quinic acid (**19**) showed potent inhibition with IC_{50} value 0.0467 ± 0.0018 as compared to standard ascorbic acid and BHT (Table 1). On the basis of these results it is found that all parts extracts, fractions and their sub-fractions showed moderate to high antioxidant activities, while pure isolated compounds were found to be very weak antioxidants except 5-*O*-caffeoyl quinic acid (**19**) which was isolated from the active fraction of *I. coccinea* flowers and might be responsible for promising antioxidant activity of flowers.

The 70% methanolic extract of fruits of *A. marmelos* (AMFM) exhibited the antioxidant activity with IC_{50} value 0.0933 ± 0.002 mg/mL and significant difference between the inhibitory effects at different concentrations was observed ($P < 0.05$). The fractions AMFMEA, AMFMBut and AMFMM were also showed antioxidant property with IC_{50} values 0.0678 ± 0.0012, 0.2525 ± 0.017 and 0.081 ± 0.001mg/mL. The sub-fractions of ethyl acetate fraction AMFMEAHEA, AMFMEAEA, AMFMButM, AMFMMHEA, AMFMMEA and AMFMMM were found to be active with IC_{50} values 0.0578 ± 0.002, 0.1076 ± 0.0007, 0.1437 ± 0.001, 0.0794± 0.0038, 0.0495± 0.0005 and

0.0936± 0.0018 mg/ ml respectively. However, the isolated pure compound marmelosin (**26**) and white crystalline compound (unidentified) were found to be inactive at the concentration of 0.5 mg/ mL as compared to the standard drugs ascorbic acid and BHT (Table 1).

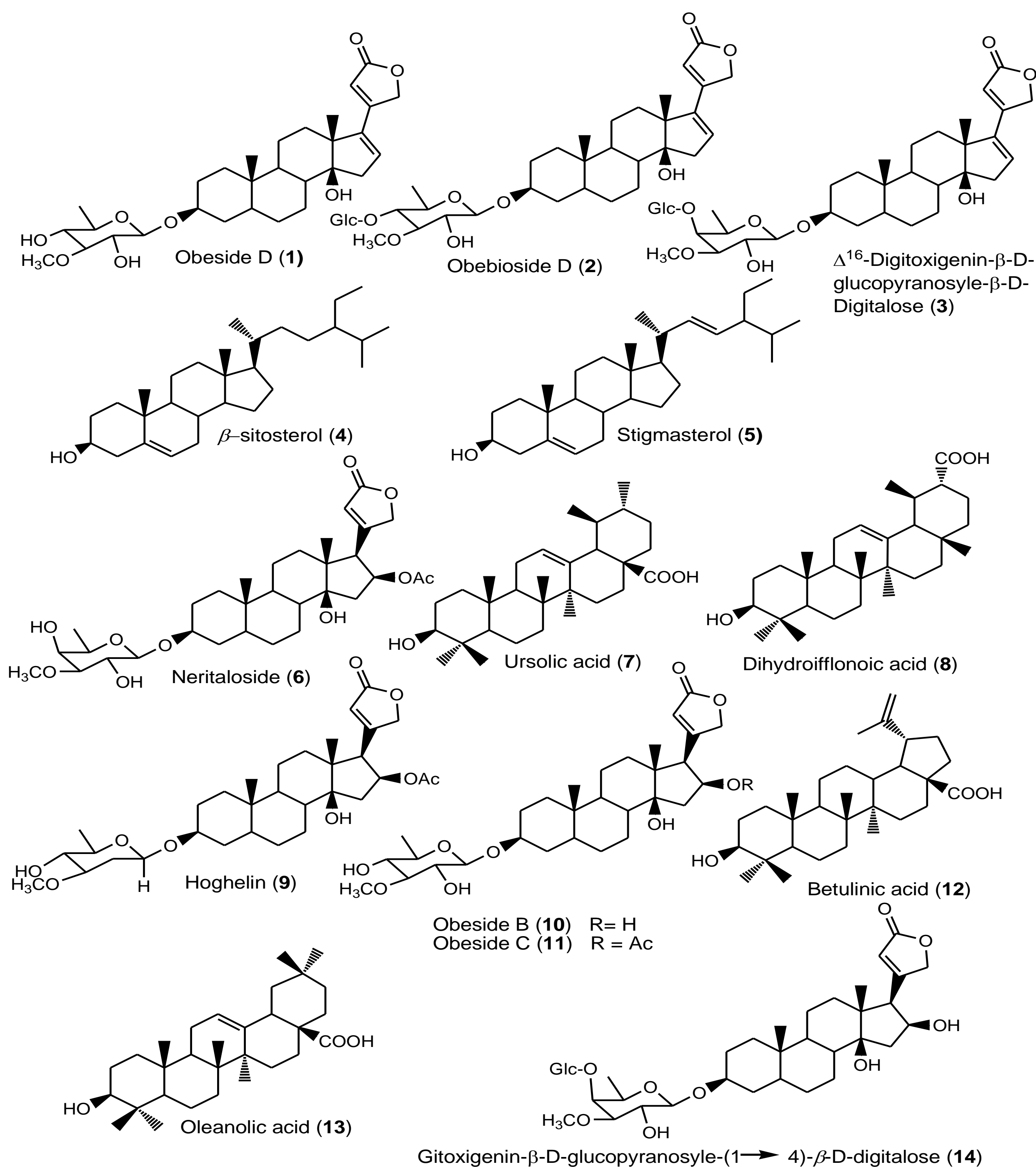

Fig.1. Structures of the compounds isolated from *Adenium obesum* (Forssk.) Roem. & Schult.

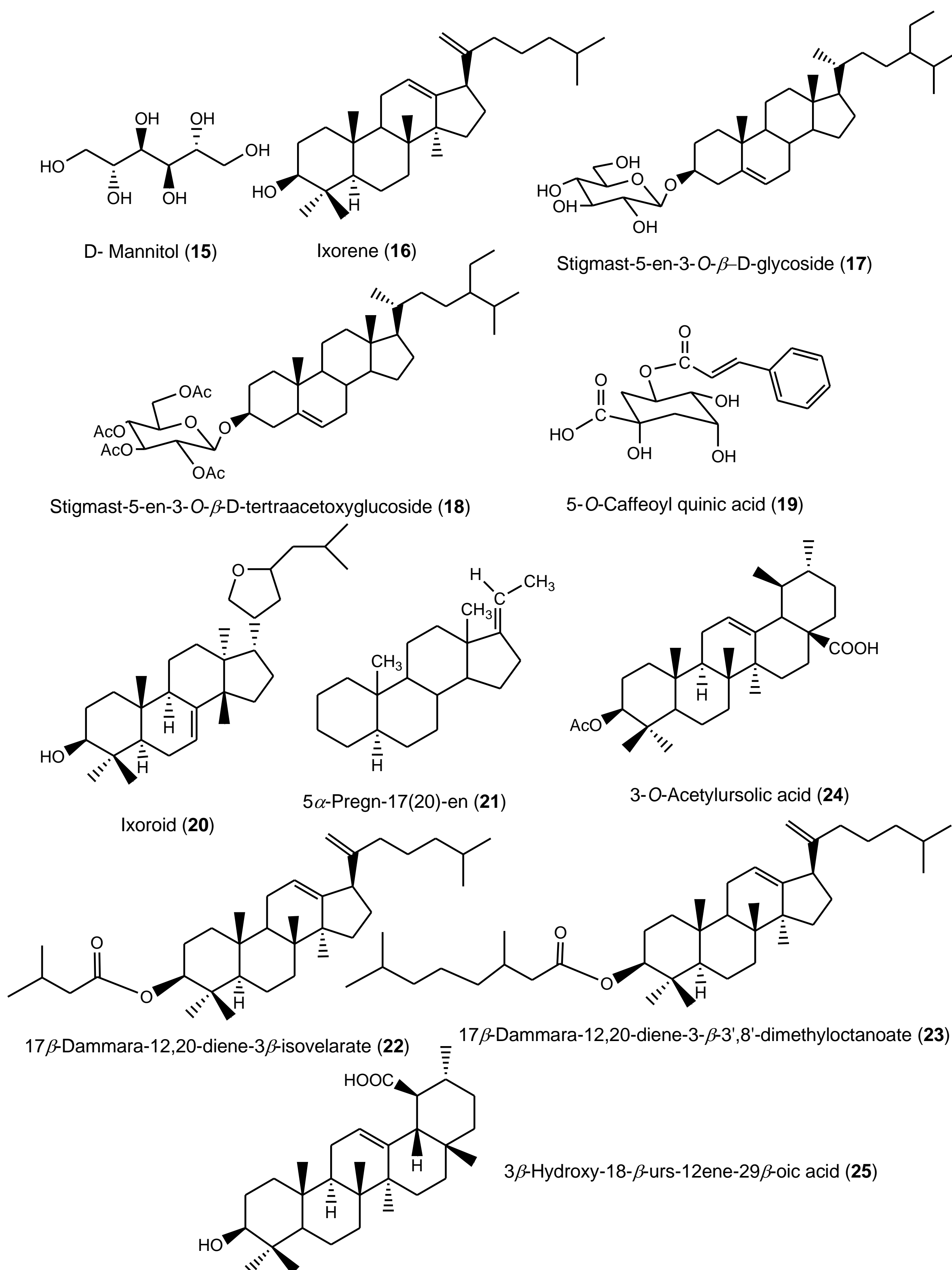

Fig.2. Structures of the compounds isolated from *Ixora coccinea* Linn

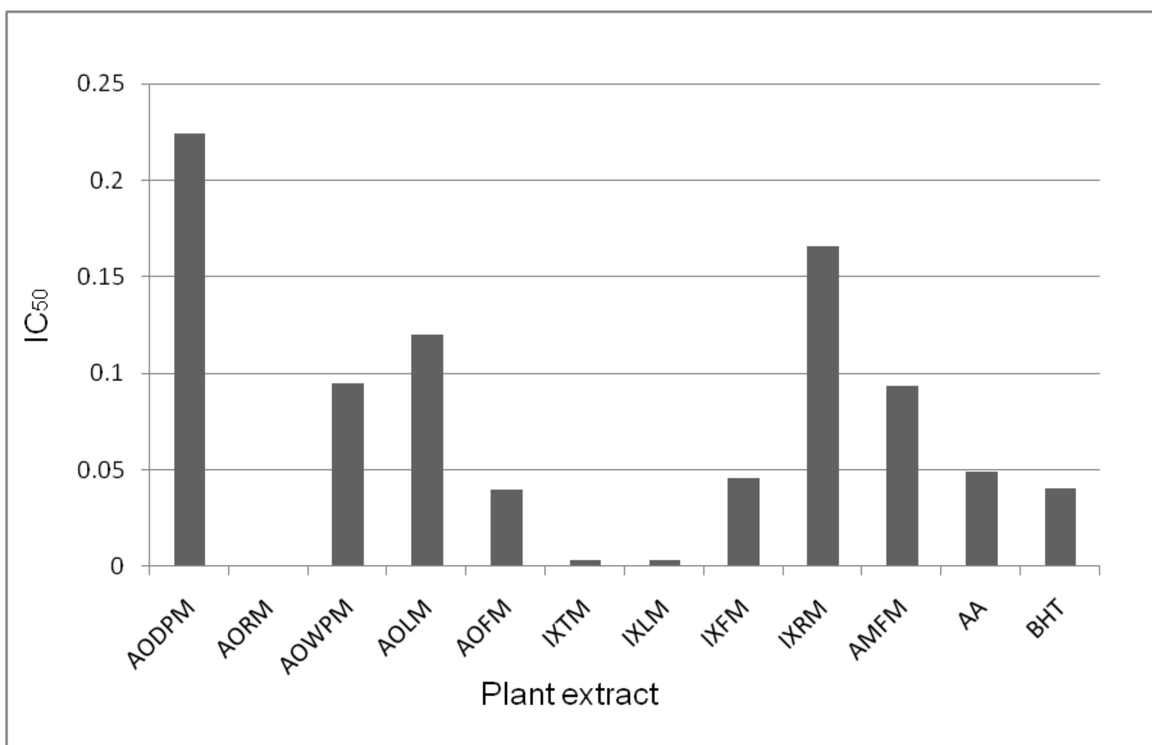

Fig.3. Antioxidant capacity of plant extracts in comparison with A.A and BHT. Lower IC_{50} value indicates higher antioxidant activity.

AODPM = *A. obesum* methanolic extract of dry pod; AOWPM = *A. obesum* methanolic extract of wet pod
AORM = *A. obesum* methanolic extract of root; AOLM = *A. obesum* methanolic extract of leaves;
AOFM = *A. obesum* methanolic extract of flowers; IXTM = *I. coccinea* methanolic extract of twigs;
IXLM = *I. coccinea* methanolic extract of leaves; IXFM = *I. coccinea* methanolic extract of flowers;
AMFM = *A. marmelos* methanolic extract of fruits; A.A = Ascorbic acid
BHT = Butylated hydroxyl toluene

Conclusion

On the basis of above results it is concluded that selected medicinal plant extracts, fractions and most of the sub-fractions showed moderate to high antioxidant activities while the pure isolated compounds from these fractions were found to be very weak antioxidants, except 5-*O*-caffeoyl quinic acid (**19**) which was isolated from the active fraction of *I. coccinea* flowers and exhibited the potent antioxidant activity towards DPPH free radical. The antioxidant capacity of these plants revealed due to the emergence of bioactive composition, which are promising source of natural antioxidants and can be exploited for multiple industrial and domestic applications.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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Table 1 ***In-vitro*** **antioxidant activity of extracts, fractions, sub-fractions and pure isolated constituents from** ***A. obesum, I. coccinea*** **and** ***A. marmelos***

Name of plant	Extract/Fractions/Pure compounds	% Inhibition (0.5 mg/mL)	IC_{50} ± SD (mg/mL)
Adenium obesum	**Extracts**		
	AODPM	80.42	0.2242±0.0020
	AOWPM	85.00	0.0951±0.0030
	AORM	16.84	-
	AOLM	87.40	0.1198±0.0107
	AOFM	89.84	0.0395±0.0023
	Fractions/ Sub-fractions of flower		
	AOFMM-70	73.88	0.0584±0.0015
	AOFMM	79.30	0.2752±0.0001
	AOFMBut	44.56	-
	AOFMEA	63.22	0.1502±0.0029
	Pure isolated constituents from flower		
	Obeside D (**1**)	0.48%	-
	Obebioside D (**2**)	6.53%	-
	Δ^{16}-Digitoxigenin-β-D-glucopyranosyle-β-D-Digitalose (**3**)	1.06%	-
	β-sitosterol (**4**)	30.24%	-
	Stigmasterol (**5**)	16.92%	-
	Pure isolated constituents from pods		
	Neritaloside (**6**)	1.69%	-
	Ursolic acid (**7**)	13.55%	-
	Dihydroifflonic acid (**8**)	0.58%	-
	Honghelin (**9**)	1.44%	-
	Obeside B (**10**)	12.60%	-
	Obeside C (**11**)	8.59%	-
	Betulinic acid (**12**)	6.21%	-
	Oleanolic acid (**13**)	41.31%	-
	Gitoxigenin-β-D-glucopyranosyle-(1→4)-β-D-digitalose (**14**)	0.08%	-
Ixora coccinea	**Extracts**		
	IXTM	97.009	0.0035±0.0003
	IXLM	86.880	0.0032±0.0001
	IXFM	93.158	0.0459±0.0027
	Fractions of leaves		
	$IXLM_1$	96.422	0.0023±0.0001
	$IXLM_2$	84.535	0.1881±0.0007
	$IXLM_3$	9.1720	-
	$IXLM_4$	93.514	0.0016±0.0004
	$IXLM_5$	91.050	0.0267±0.0016
	$IXLM_6$	88.688	0.0130±0.0057
	Fractions of flowers		
	IXFMAq	92.184	0.0491±0.0010
	IXFMEA	89.3260	0.0048±0.0004
	Sub-fractions of flowers		
	IXF_1	84.8770	0.0061±0.0001
	IXF_2	88.0800	0.0032±0.0001
	IXF_3	95.0460	0.0015±0.0003

	IXF_4	92.4990	0.0023±0.0004
	IXF_4b	95.2495	0.0302±0.0014
	IXF_1BA	85.7788	0.0652±0.0008
	IXF_1BB	94.1080	0.0615±0.0001
	IXF_1BC	98.3991	0.0478±0.0001
	Pure isolated constituents from leaves		
	D-mannitol (**15**)	6.6020	-
	Ixorene (**16**)	4.9095	-
	Pure isolated constituents from flowers		
	Stigmast-5-en-3-*O*-*β*-D-glycoside (**17**)	2.890	-
	Stigmast-5-en-3-O-β-D-tetra-acetoxy glycoside (**18**)	2.048	-
	5-*O*-Caffeoyl quinic acid (**19**)	77.031	0.0467 ±0.0018
	19, 21-Epoxy-tirucall-7-en-3-ol (Ixoroid, **20**)	5.681	-
	5*α*-Pregn-17-en (**21**)	5.133	-
	17-*β*-Dammara-12,20-diene-3-*β*-isovelarate (**22**)	5.941	-
	17-*β*-dammara-12,20-diene-3-*β*-3',8'-dimethyloctanoate (**23**)	5.502	-
	3-Acetoxy ursolic acid (**24**)	2.8177	-
	3-*β* -Hydroxy-18-*β*-urs-12ene-29*β*-oic acid (**25**)	7.097	-
Aegle marmelos	**Extract of fruit**		
	AMFM (70% methanol)	78.087	0.0933 ± 0.002
	Fractions of fruit		
	AMFMEA	94.000	0.0678 ± 0.0012
	AMFMBut	84.499	0.2525 ± 0.0170
	AMFMM	76.570	0.0810 ± 0. 0010
	Sub-fractions of fruit		
	AMFMEAHEA	93.421	0.0578 ± 0.0020
	AMFMEAEA	93.384	0.1076 ± 0.0007
	AMFMEAM	57.150	-
	AMFMButEA	65.214	-
	AMFMButM	80.000	0.1437 ± 0.0010
	AMFMMH	42.4670	-
	AMFMMHEA	83.1276	0.0794±0.0038
	AMFMMEA	87.4627	0.0495±0.0005
	AMFMMM	78.8749	0.0936±0.0018
	Pure isolated constituents from fruit		
	Marmelosin (**26**)	3.4971	-
	Unidentified Compound (white crystals)	8.7895	-
	Positive control		
	Ascorbic acid	96.100	0.0491 ± 0.0009
	Butylated hydroxytoulene (BHT)	75.700	0.0407 ± 0.003

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