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Comparative phytochemical and biological studies of lipoidal matter of Ipomoea tricolor (Cav.) and Ipomoea fistulosa (Mart. Ex Choisy) growing in Egypt

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Abstract

Lipoidial profile of *Ipomoea tricolor* and *Ipomoea fistulosa* leaves were characterized by GC/MS led to the identification of 22 and 33 compounds represented (95.88 and 91.82 %), respectively, of *I. tricolor* and *I. fistulosa* unsaponifiable matter It is composed mainly of hydrocarbons and oxygenated compounds. The GC/MS of fatty acids methyl ester (FAME) led to the identification of 8 and 10 compounds for *I. tricolor* and *I. fistulosa*, respectively.Linolenic and palmatic acids were found as the predominant fatty acids. On the other hand, the petroleum ether extract of *I.tricolor* was fractionated on column chromatography to yield major compounds; 5-phenyldecane and 4-phenyldecane, as well as, triterpenes including *β*-amyrin, lupeol and simiarenol,in addition to *α*- amyrin, *β*-sitosterol, stigmasterol and *α*- amyrin acetate which isolated for the first time from this species.The petroleum ether extracts of *I. tricolor*and *I. fistulosa* showed a significant increase in DPPHinhibition activity at 0.01 and 0.05 ug/ml, respectively. Furthermore, *I.tricolor* extract exhibited a significant increase in cholinesterase inhibition activity at concentration 20 ug/ml and a significant anti-inflammatory activity with IC_{50} 117.4µg/ml. It can be concluded that pet.ether extracts of *I.tricolor* and *I.fistulosa* at different concentrations have significant antioxidant, anticholinesterase and anti-inflammatory activities related to various bioactive identified compounds.

Keywords: *Ipomoea tricolor*, *Ipomoea fistulosa*, triterpenes, antioxidant, anticholinesterase ,ant-inflammatory.

1. Introduction

Family Convolvulaceae which known also as a Morning Glory plants constitute a total 57 genera and about 1600 species [1]. This family is dominated by twining or climbing woody or herbaceous plants that often have heart-shaped leaves and funnel-shaped flowers[2]. Genus *Ipomoea* comprises of about 600-700 species, which are widely distributed throughout tropical and subtropical regions of the world it had been used traditionally to treat many diseases including, diabetes, hypertension, dysentery, fatigue, inflammations, arthritis, rheumatism, constipation ,hydrocephaly, meningitis and kidney ailments [3]. Several of those species have been used as medicines, food, ornamental plants and or in religious ritual [3] .

Ipomoea tricolor one member of family Convolvulaceae had the property of phytotoxicity, which means suppressing the growth of other plants including invasive weeds [4]. Previous studies reported the isolation of resin glycosides including tricolorins A-E as a natural tetrasaccharide macro lactones and linear tetraglycosides of jalapinolic acid from *I.tricolor* which have strong herbicidal properties [4]. Tricolorin a showed strong herbicidal and antimicrobial activities as well as cytotoxic activity against human breast cancer, colon carcinoma, squamous cell cervix carcinoma, ovarium cancer cell lines and oral epidermoid carcinoma [5]. Two oxylipins, cis-12-oxophytodienoic acid and

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monogalactosyl-monoacylglyceride were previously isolated which acted as inducers of stomatal opening [6]. Several ergoline alkaloids were isolated from *I. tricolor* which increase the motor activity of the uterus as ergotamine which presented also vasoconstrictor activity and was useful in the treatment of migraine headaches [3].

Other specie was *Ipomoea carnea spp. fistulosa* (Mart. ex Choisy) *D.Austin* which also known as *Ipomoea carnea Jacquin* [7]. Various bioactive compounds isolated from different parts of *I. fistulosa*, Abdallah [8] extract different volatile, lipid constituents and flavonoids from different parts of plant by using different methods hydrodistillation (HD), microwave (MAE), solvent extraction (SE) and solid-phase-microextraction (SPME) and all of them were analyzed using GC/MS and their antioxidant activity was evaluated in addition the anti-inflammatory activity of pet. ether extract was evaluated by Ruch [9]and attributed the activity mainly to the presence of β-sitosterol. Kamal [10] studied the ethanol extract and isolated ipomoeflavoside, caffeoyl ethyl ester, caffeic acid, rutin, lycopene from leaves and β-sitosterol from flowers and evaluate their antioxidant, antihyperglycemic, hepatoprotective, anticancer and antimicrobial activities . Gaur [11] reported that methanolic leaves extract exhibited significant antioxidant activity in comparison with standard (BHT). Furthermore Haraguchi [12] isolated polyhydroxylated alkaloid from the leaves , *i.e.*, swainsonine, 2-epi-lentiginosine ,calystegines B1, B2, B3, and C1, and N-methyl-trans-4-hydroxy-L-proline and studied their inhibitory activity toward lysosomal, β-glucosidas, α- mannosidases and β-mannosidases. Moreover, Abdel Sattar [13] isolated 3-caffeoylquinic acid (clorogenic acid) and 3, 5-dicaffeoylquinic acid esters from the seeds .

The main target of the presented work was to reach safe and effective therapeutic agents from natural origin which can be useful in drug industry. So we targeted to identify major phytoconstituents from the bioactive fractions as few studies applied on lipoidal matter of *I. tricolor* and *I. fistulosa* that's encouraging applying GC/MS analysis to reveal the components of the saponifiable, unsaponifaible matters and major fractions of pet. ether extract in addition to isolate major compounds and identification of them by using different chromatographic techniques and evaluate their antioxidant, anticholinesterase and anti-inflammatory activities.

Experimental: Plant material

The fresh plant *Ipomoea tricolor* (Cav.) and *Ipomoea fistulosa* (Mart. Ex Choisy) leaves were collected from El Qanater El Khayreya of El Qalyubiy Governorate, Egypt. April 2016 during flowering stage. The plant materials were identified by Mrs. Threase Labib consultant of plant taxonomy at the Ministry of Agriculture. A voucher specimens (No.31-8-2016 I) and (No.31-8-2016 II) for *I. fistulosa* and *I. tricolor* respectively were kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University. The collected leaves were air dried (2 Kg), separately powdered and kept in tightly-closed containers.

Chemicals

All chemicals are of high analytical grade, products of Merck, Germany and Sigma, USA. Nuclear magnetic resonance (NMR) experiments were recorded on a Bruker spectroscopy: 500 MHz (1H NMR). Gas chromatography/mass spectrometry analysis (GC/MS) analysis was carried out using a Finnigan SSQ 7000 (ThermoFinnigan, San Jose, California, USA) GC/MS spectrophotometer equipped with library software Wiley 138 and NBS 75 under the following conditions: DB-5-fused silica capillary column, 30 m in length, 0.32 mm ID, and with a film thickness of 0.25 μm; carrier gas, helium at a flow rate of 10 ml/min; temperature programmed to 60–260°C at a rate of 4°C/min, chart speed: 0.5 cm/min, ionization voltage 70 eV, and detector: flame ionization detector

Preparation of extract

The dried powder (2Kg) was extracted with absolute methanol and dried using rotavapour at 40 °C. The residual extract was fractionated with pet. ether(60-80). Then the prepared extracts were evaporated to dryness under vacuum at 40°C, weighted and were ready for biological and chemical examination.

Investigation of lipoidal matter

Preparation of unsaponifiable matters and saponifiable matter as well as FAME were performed according to Matloub [14] then subjected to GC/MS analysis on Shimadzu GC/MS–QP5050A. Identification of the constituents has been carried out by comparison of their spectral fragmentation patterns with those of the available database libraries [Wiley (Wiley Int.) USA and NIST (Nat. Inst. St. Technol., USA)] and/or published data by Adams [15] Quantitative determination was carried out based on peak area integration .From unsaponifiable matters we

isolated two major compounds (1 and 2) and identify them by using different spectral analysis.

Column analysis

Isolation and purification of the main active fractions, (12) gm petroleum ether extract of *I.tricolor* leaves were subjected to column chromatography (120 cm height x 2.5 cm i.d.) and the elution was carried out by petroleum ether (60-80°C) and increasing polarity by methylene chloride. Fractions each of 100 ml were successively collected, concentrated to 5 ml and screened by TLC, using benzene : ethyl acetate (8:2, 7:3 and 86:14 and v/v) as solvent system, and sulfuric acid reagent (20% in ethanol) used for detecting terpenoids The resulting similar fractions pooled together according to R_f values afford three main sub fraction I (100% petroleum ether), fraction II (60% pet.ether / dichloromethane) and fraction III the fractions I and II subjected to GC/MS to identify the major compounds and two major compounds were isolated (3 and 4) from fraction III (40% petroleum ether) identify them by using different spectral analysis .

Biological study

The antioxidant study using DPPH• free radical scavenging activity

Quantitative measurement of free radical scavenging properties of pet.ether extracts from *I. tricolor* and *I.fistulosa* was carried out according to the method [16] which stated that 0.1ml solution of 1, 1- diphenyl-2-picryl-hydrazyl (DPPH·) was prepared in 100 ml absolute methanol and 1 mL of this solution was added to 1 mL of each extract sample and vitamin c (reference drug) at three concentrations (0.01, 0.05 and 0.1 µg/ml). Discoloration was measured at 517 nm after incubation for 30 min. Measurements were taken at least in triplicate. The scavenging ability of DPPH• was calculated using the following equation: Scavenging effect (%) = $A0-A1A0\times100$

Where *A0* is the absorbance of DPPH• solution (without the tested extract) and *A1* is the absorbance of the tested extracts with DPPH• solution.

Assay of acetyl cholinesterase (AChE) enzyme activity by the spectrophotometric method

AChE activity was measured by using spectrophotometer based on Ellman's method [17] The enzyme hydrolyzes the substrate acetylthiocholine resulting in the product thiocholine which reacted with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 412 nm. In test tube 1710 µl of 50 mM Tris–HCl buffer pH 8.0 and 250 µl of extact samples of tested microalgae and standard drug at two concentrations of 10 and 20 µg/ ml, 10 µl 6.67 UmL-1 AChE and 20 µl of 10 mM of DTNB (5,5'-dithio-bis[2-nitrobenzoic acid]) in buffer were added. Positive control namely dopenzil was prepared in serial concentration as same as tested samples by dissolving in 50 mM Tris–HCl buffer pH 8.0. The mixture was incubated for 15 min at 37 ºC. Then, 10 µl of acetylthiocholine iodide (200 mM) in buffer was added to the mixture and the absorbance was measured at 412 nm every 10 sec for 3 min. For a blank, the buffer instead of enzyme solution was used. The enzyme inhibition (%) was calculated from the rate of absorbance change with time (V= Abs/Δt) according to calculation as follows:

Inhibition (%) = 100 - Change of sample absorbance X 100 change of blank absorbance

The experiment was done in triplicate for each concentration of the tested samples that inhibit the hydrolysis of the substrate (acetylcholine). The percent of acetylcholinesterase inhibition was calculated as follows: % Inhibition = 100 − [Absorbance of the test extract/Absorbance of the control] × 100.

Anti-inflammatory activity using histamine release Assay

U937 human monocytes (ATCC, Manassas, VA, USA) were used to study the effect of samples on histamine release according to Venkata [18], approximately 50,000 U937 cells were plated in a 96-well cell culture plate (Corning Life Sciences, Lowell, MA, USA) and treated with various concentration (1000-7.81µg/ml) of samples, in presence or absence of 20 nM Phorbol Myristate Acetate (PMA) (Sigma-Aldrich, St. Luis, MO, USA) for one hour. The cell culture supernatants collected from either untreated control or treated cultures were clarified at 10,000 g for 5 min at 4°C and assessed for released histamine by commercially available EIA kit (SPI-Bio, France).

Results

The yield of pet. ether extracts were (5.3 and 4.52% w/w of dried powder) for *I. tricolor* and *I. fistulosa* leaves respectively .Saponification of the pet. ether extract of both tested extracts was afforded (65.71and 63.23 w/w%) of unsaponifiable matter, respectively .The results of GC/MS analysis of the USM of *I. tricolor and I. fistulosa* represented in Table (1) revealed the identification of 22 compounds represented (95.88%) of the total USM of *I.tricolor* and 33 compounds represented (91.82%) of the total USM of *I.fistulosa* . The results revealed a high

relative percentage of non-oxygenated compounds consist mainly of a mixture of hydrocarbons from C_{10} to C_{19} hydrocarbons (86.94 and 90.38%) for *I. tricolor and I. fistulosa*, respectively, among which, the major constituents were 5-phenyltridecane (21.11%), 2-pdenylundecane (14.69%) and 2-phenyldodecane (8.07%) *of I.tricolor and* 5-phenylundecane(12.19%), 2-phenylundecane (8.77%) and 4-phenylundecane (9.79%) were the main constituents of *I.fistulosa*. The identified oxygenated compounds representing (8.94 and 1.44%) for *I.tricolor* and *I.fistulosa* , respectively,. Whereas the phytol identified only in the USM of *I.tricolor* represented (8.94%). previous study stated that hexane fraction of *I.carnea* yielded 2.70% of oil which analyzed by GC/MS, indicates presence of 13 compounds included hexadecanoic acid, stearic acid, 1,2 diethyl phthalate, n-octadecanol, octacosane, hexatriacontane, tetraacontane, 3- diethylamino-1 propanol [19], Abdallah [8] extracted the volatile constituents of different organs (flowers, leaves and stems) of *I. carnea* by using different methods (hydrodistillation (HD), microwave (MAE), solvent extraction (SE) and solid-phase-microextraction (SPME) and all of them were analyzed using GC/MS.

Table 1: Compounds identified by GC/MS analysis of the USM of *Ipomoea tricolor* and *Ipomoea fistulosa*

RRt	B.P	M.F	M.WT	Main fragments	Compound	Area%	
						I.t	*I.f*
0.33	57	$C_{10}H_{20}$	140	97 ,67,81,111	1-Decene	-	0.35
0.34	57	$C_{10}H_{22}$	142	98,85,113,71	Decane	-	1.82
0.38	83	$C_{10}H_{20}$	140	55,99,111	Butylcyclohexane	-	0.38
0.41	57	$C_{11}H_{22}$	154	83,99,71,139	1-Undecene	-	0.96
0.44	83	$C_{11}H_{22}$	154	83,55,97	Pentylcyclohexane	-	0.42
0.45	57	$C_{11}H_{24}$	156	71,85,99,113	Undecane	2.31	4.93
0.47	152	$C_{10}H_{16}O$	152	67,81,95,137,110	Pulegone	-	0.99
0.49	83	$C_{12}H_{24}$	168	83,55,97	Hexylcyclohexane	-	1.12
0.51	43	$C_{12}H_{26}$	170	57,71,85	4-Methylundecane	-	0.56
0.52	57	$C_{12}H_{26}$	170	71,85,113,155	3-Methylundecane	-	0.97
0.53	43	$C_{12}H_{26}$	170	57,71,85 ,155	2-Methylundecane	-	0.66
0.54	97	$C_{11}H_{22}O$	170	67,82,152	Undecanal	-	0.45
0.55	57	$C_{12}H_{26}$	170	71,85,113,127	Dodecane	2.83	5.73
0.56	57	$C_{13}H_{28}$	184	71,85,98,112	Undecane, 2,6-dimethyl-	-	0.88
0.59	83	$C_{13}H_{26}$	182	55,67,109,140	Heptylcyclohexane	-	0.71
0.65	57	$C_{13}H_{28}$	184	57,71,85	Tridecane	-	0.56
0.86	91	$C_{16}H_{26}$	218	105,161,189	5-phenyldecane	0.18	4.07
0.87	91	$C_{16}H_{26}$	218	105,133,175	4- phenyldecane	0.31	3.52
0.88	91	$C_{16}H_{26}$	218	189,105,119,147	3-phenyldecane	0.60	_
0.90	105	$C_{16}H_{26}$	218	79,91,105	2-phenyldecane	1.02	5.71
0.91	91	$C_{17}H_{28}$	232	175,118,161	6-phenylundecane	2.54	0.42
0.94	91	$C_{17}H_{28}$	232	189,175	5-phenylundecane	1.61	12.19
0.95	91	$C_{17}H_{28}$	232	105,147,161,190	4-phenylundecane	4.52	6.79
0.96	91	$C_{17}H_{28}$	232	203,188,161	3-phenylundecane	6.34	6.59
1	105	$C_{17}H_{28}$	232	119,133,147	2-phenylundecane	14.69	8.77
1.01	91	$C_{18}H_{30}$	246	175,105,119,161	6-Phenyldodecane	2.93	2.80
1.02	91	$C_{18}H_{30}$	246	189,105,147	5-Phenyldodecane	3.03	2.76
1.03	91	$C_{18}H_{30}$	246	203,105,133	4-Phenyldodecane	2.99	
1.04	91	$C_{18}H_{30}$	246	216,105,119,161	3-Phenyldodecane	3.83	2.58
1.07	105	$C_{18}H_{30}$	246	91,231,189	2-Phenyldodecane	8.07	4.34
1.08	91	$C_{19}H_{32}$	260	189,105,161,174	6- phenyltridecane	2.21	2.19
1.09	91	$C_{19}H_{32}$	260	203,105,147	5- phenyltridecane	21.11	2.05
1.10	91	$C_{19}H_{32}$	260	216,105,133,161	4-phenyl Tridecane	1.97	1.29
1.11	91	$C_{19}H_{32}$	260	231,105,119	3-phenyl Tridecane	-	2.13
1.12	81	$C_{20}H_{40}O$	296	278,260,123,95	Phytol	8.94	-
1.14	105	$C_{19}H_{32}$	260	91,147,161,189, ,218	2-phenylTridecane,	3.85	2.31
Area Total identified compounds %						95.88	91.82
Hydrocarbon						86.94	90.38
Oxygenated compound						8.94	1.44

I.t:Ipomoea tricolor *I.f*: Ipomoea fistulosa RRt: Relative Retention time; relative to (2-phenylundecane) 29.38
M.Wt.: Molecular weight M.F.: Molecular formula B.P: base peeak

FAME was prepared by methylation process of the saponifiable matter yielded (34.29 and 36.77 w/w % of the total extracts) for *I.tricolor and I. fistulosa* ,respectively,. The results of GC/MS analysis of FAME (Table: 2) revealed identification of 8 and 10 compounds for *I.fistulosa* and *I. tricolor*

respectively and the total relative percentage of identified saturated fatty acids was high in *I.fistulosa* than *I. tricolor* contributed with (66.92%) and (38.63%) ,respectively ,where palmitic acid methyl ester represented the major in both (52.33%) and (31.12%) respectively, then stearic acid methyl ester (7.92%) and (7.16 %) respectively for *I.fistulosa* and *I.tricolor* while the unsaturated fatty acids represented high relative percentage in *I.tricolor* than *I.fistulosa* (61.37%), (33.08%), respectively, where linolenic acid methyl ester represented the major in both (34.80%) and (24.56%) respectively. These results matched with[8] who stated that saturated fatty acids of *I. fi*stulosa was (81.91%) and the major fatty acids were methyl palmitate and methyl stearate but not agree with our study as they reported that unsaturated fatty acids comprise monounsaturated fatty acid (11.63%), diunsaturated (6.46%) and absence of polyunsaturated fatty acid . Palmatic and stearic acids isolated before from *I. cairica* and determined their Cytotoxic activity by [20] Our study considers the first study GC/MS investigation of saponifiable and unsaponifiable matters of *I.tricolor species*.

The GC/MS analysis of unsaponifiable matter didn't reveal triterpenes or sterol compounds that led us to fractionate the pet. ether extract of *I.tricolor* over column chromatography of silica gel . The main fraction I (8.7%w/w) eluted with 100% petroleum ether and II (6.32%w/w) eluted with 60% petroleum ether / dichloromethan) and further subjected to GC/MS. The results in (Table:3) revealed that fraction I yielded 37 compounds consisted mainly of a high relative percentage of non-oxygenated compounds (81.43%) mainly was a mixture of hydrocarbons from C_{10} to C_{25} with major compounds 5-phenyldecane (12.5%) and 2,4,6 trimethyl heptane (9.24%), sesqueterpens (9.2%) included trans caryophyllene (0.68%) β- gurjunene (2.73%), α-guaiene (2.56%) and β-cubebene (3.23%). The oxygenated compounds represented (10.04%) included fatty acid methyl ester (7.95%) and the major was methyl linolenate (2.10%).

Table 2 : FAME identified by GC/MS analysis of the S.M of *Ipomoea tricolor* and *Ipomoea fistulosa* .

RRt	Identified Compound		M.F	M.WT	B.P	Relative area	
						I.t	*I.f*
1	Palmitic acid methyl ester	C16:0	$C_{17}H_{34}O_2$	270	74	31.12	52.33
1.2	Oleic acid methyl ester	C18:1	$C_{19}H_{36}O_2$	296	55	0.31	4.68
1.24	Linoleic acid	C18:2	$C_{19}H_{34}O_2$	294	67	26.26	3.84
1.25	linolenic acid methyl ester	C18:3	$C_{19}H_{32}O_2$	292	67	34.80	24.56
1.27	Stearic acid methyl ester	C18:0	$C_{19}H_{38}O_2$	298	74	7.16	7.92
1.51	Arachidic acid methyl ester	C20:0	$C_{21}H_{42}O_2$	326	74	0.18	1.86
1.74	Behenic acid methyl ester	C22:0	$C_{23}H_{46}O_2$	354	74	0.09	1.44
2.15	Lignoceric acid methyl ester	C24:0	$C_{25}H_{50}O_2$	382	74	0.08	1.86
2.17	Methyl hexacosanoate	C26:0	$C_{27}H_{54}O_2$	410	74	--	0.91
2.36	Methyl octacosanoate	C28:0	$C_{29}H_{58}O_2$	438	74	--	0.6
Percentage of total saturated FAME						38.63	66.92
Percentage of total unsaturated FAME						61.37	33.08

RRt=Relative retention time and Relative area % is relative to Palmitic acid* (Rt= 16.8 min)
M.Wt.: Molecular weight M.F.: Molecular formula B.P: base peak

Table 3: GC/MS analysis of the fraction I from the column of petroleum ether of *Ipomoea tricolor* leaves

RT	B.b	M.F	M.WT	Main fragments	Compound	Area%
14.19	43	$C_{10}H_{22}$	142	85,55,69, 98	2,4,6Trimethylheptane	9.24
14.54	57	$C_{12}H_{26}$	170	71,85,112,99	Dodecane	1.25
15.90	57	$C_{13}H_{28}$	184	71,85,97, 141	2,10Dimethylundecane	1.87
16.14	57	$C_{14}H_{30}$	198	71, 85,97, 112	7-Methyltridecane	2.07
16.90	57	$C_{13}H_{28}$	184	71, 85, 99	Tridecane	5.85
19.28	55	$C_{14}H_{28}$	196	70, 97, 84, 111	1-tetradecene	3.92
19.49	43	$C_{14}H_{30}$	198	57,71, 99, 85, 113	Tetradecane	8.64
20.08	41	$C_{15}H_{24}$	204	93,69,81,106,161	Trans Caryophyllene	0.68
20.49	74	$C_{11}H_{22}O_2$	186	55, 87,111,143	Methyl caprate	0.69
20.6	91	$C_{15}H_{24}$	204	147,105	5-phenylnonane	0.84
20.72	91	$C_{15}H_{24}$	204	105, 119,133, 161	4-phenylnonane	1.23
21.12	91	$C_{15}H_{24}$	204	105,119,133,175	3-phenylnonane	1.46
21.50	41	$C_{15}H_{24}$	204	79,105,119,161,189	β- Gurjunene	2.73
21.95	105	$C_{15}H_{24}$	204	119,147,175,77,55	α Guaiene	2.56
22.41	105	$C_{15}H_{24}$	204	105,161,119,133	β-Cubebene	3.23
22.54	191	$C_{14}H_{22}O$	191	191,207	2,4Diterbutylphenol	1.37
22.63	91	$C_{16}H_{26}$	218	91,105,153,175	6-phenyldecane	5.31
22.91	91	$C_{16}H_{26}$	218	105,133,147,119,161	5-phenyldecane*	12.5
23.14	91	$C_{16}H_{26}$	218	105,119,133,175	4-phenyldecane	5.35
23.32	105	$C_{16}H_{26}$	218	79,147,203	2-phenyldecane	2.18
23.56	119	$C_{18}H_{30}$	246	91,104,133	(1,1,4,6,6)Pentamethylheptyl)benzene	2.49
23.82	91	$C_{17}H_{28}$	232	133,189,104	6-phenylundecane	0.64
23.98	91	$C_{17}H_{28}$	232	119,189,161	5-phenylundecane	1.25
25.79	91	$C_{17}H_{28}$	232	133,,119,189	4-phenylundecane	0.75
26.28	91	$C_{17}H_{28}$	232	119,133,147	3-phenylundecane	0.72
27.79	91	$C_{18}H_{30}$	246	119,189,147	5-phenyldodecane	0.61
28.48	91	$C_{18}H_{30}$	246	119,133,161	3-phenyldodecane	0.54
33.04	74	$C_{18}H_{36}O_2$	284	143,241,199	Methyl margarate	0.52
34.28	55	$C_{15}H_{26}O_2$	238	67,82,95	Methyl10,11tetradecadienoate	1.87
34.43	55	$C_{19}H_{32}O_2$	292	80,93,108	Methyl linolenate	2.10
34.38	74	$C_{17}H_{32}O_2$	268	87,143,199	Methyl palmitoleate	1.37
35.90	55	$C_{22}H_{44}$	308	83,97,111,139,280	1-Docosene	1.40
37.62	57	$C_{23}H_{48}$	324	71,85,99,113,267	Tricosane	0.71
38.17	74	$C_{21}H_{42}O_2$	326	87,199,283	Eicosanoic acid methylester	1.40
39.15	43	$C_{24}H_{48}$	336	83,97,111	Cyclotetracosane	0.71
40.47	57	$C_{25}H_{52}$	352	71,85,99	Pentacosane	0.70
42.17	97	$C_{24}H_{50}O$	354	83,111,125	n-Tetracosanol	0.72

Total identified compounds 91.47%

Oxygenated compound 10.04% including Fatty acid methyl ester 7.95

Hydrocarbon 81.43% including Sesquterpenes 9.2%

RT:retention time M.Wt.: Molecular weight M.F.: Molecular formula B.P: base peeak

The results in (Table: 4) revealed that fraction II yielded 36 compounds represented (90.76%) in which hydrocarbons represented (51.2%) mainly was a mixture of 3-Phenyldecane (9.88%), the oxygenated compounds represented (39.56 %) included oxygenated sesqueterpens and triterpenes represented (2.27 %) including *β*-amyrin (0.54%), lupeol(1.12%) and simiarenol(0.61%).

Table 4:GC/MS analysis of the fraction II from the column of petroleum ether of *Ipomoea tricolor* leaves.

RT	B.P	M.wt	M.F	Main fragments	Compound	Area%
14.14	57	170	$C_{12}H_{26}$	71,85,99	n-Dodecane	0.58
19.25	55	196	$C_{14}H_{28}$	69,97,125,139	1-Tetradecene	1.04
19.44	85	187	$C_{10}H_{21}NO_2$	69,99,55	Octanamide,N(2hydro-xyethyl)	2.95
21.19	135	220	$C_{15}H_{24}O$	67 ,107,149,177,205	Ionone dimethyl	0.64
21.84	119	204	$C_{15}H_{24}$	161,133,147,189,105	α-Funebrene	0.75
21.92	105	204	$C_{15}H_{24}$	147,117,189,91,79,	α –Guaiene	0.56
22.12	141	218	$C_{16}H_{26}$	91,105,153,175	6-phenyldecane	2.32
22.21	91	218	$C_{16}H_{26}$	119,105,189	5-Phenyldecane	0.68
22.37	222	222	$C_{15}H_{26}O$	161,119,105,93	Cubebol	1.43
22.51	191	206	$C_{14}H_{22}O$	57,163	2,4Diterbutylphenol	1.94
22.89	91	218	$C_{16}H_{26}$	91,105,133,175	4-Phenyldecane	12.04
23.09	105	220		119,134	14-α hydroxy cadiene	10.55
23.50	105	218	$C_{10}H_{14}$	189,105,119,147	3-Phenyldecane	9.88
23.97	43	220	$C_{15}H_{24}O$	205,119,187	Spathulenol	1.24
24.07	43	220	$C_{15}H_{24}O$	91, 69, 109,177	Caryophyllene oxide	4.05
24.33	105	218	$C_{10}H_{14}$	91,117,161	2-phenyldecane	7.45
24.45	105	218	$C_{15}H_{22}O$	77,149,177,202	1-(4-Methylphenyl) Octan-3-one	1.98
25.04	91	232	$C_{17}H_{28}$	105,119,203	3-phenylundecane	4.86
26.59	105	232	$C_{17}H_{28}$	119,133,147	2-phenylundecane	2.4
27.17	91	246	$C_{18}H_{30}$	175,105,119,161	6-Phenyldodecane	1.19
27.56	91	246	$C_{18}H_{30}$	91,203,105,133	4-Phenyldodecane	0.57
27.99	91	246	$C_{18}H_{30}$	105,119,217	3-Phenyldodecane	1.08
28.75	105	246	$C_{18}H_{30}$	91, 231,189	2-phenyldodecane	1.5
29.19	91	260	$C_{19}H_{32}$	118,161,189	6- phenyltridecane	0.78
30.06	91	260	$C_{19}H_{32}$	231,105,119,	3-phenyltridecane	1.15
30.80	105	260	$C_{19}H_{32}$	147,161,189,203,218	2-phenyltridecane	2.37
32.27	83	270	$C_{18}H_{38}O$	252,69,97,111	1-Octadecanol	0.84
33.99	83	298	$C_{20}H_{42}O$	280,69,97,111	n-Eicosanol	0.72
34.53	71	296	$C_{20}H_{40}O$	81,97,123	Phytol	0.55
35.82	43	326	$C_{22}H_{46}O$	69,125,98	1-Docosanol	1.03
38.57	99	324	$C_{21}H_{40}O_2$	114,126,151	4,8,12,16Tetramethylheptadecan-4-olide	0.51
39.10	97	354	$C_{24}H_{50}O$	97,111,125	1-Tetracosanol	0.72
41.68	149	390	$C_{24}H_{38}O_4$	113, 166	Phthalic acid, octyl Oct-3-yl ester	8.14
52.37	218	426	$C_{30}H_{50}O$	218,203,189	β-Amyrin	0.54
52.95		426	$C_{30}H_{50}O$	207,218,135	Lupeol	1.12
53.05	274	426	$C_{30}H_{50}O$	260,205,134	Simiarenol(3β-hydroxy-E:B-*friedo*-hop-5-ene)	0.61

Identified compounds represented 90.76%
Hydrocarbons 51.2%
Oxygenated compounds 39.56 %
Triterpens 2.27%

RT:retention time M.Wt.: Molecular weight M.F.: Molecular formula B.P: base peeak

Comparing the pet. ether extracts and unsaponifiable matter of *I.tricolor* and *I.fistulosa* leaves by TLC using solvent system benzene–ethyl acetate (8:2 v/v) revealed petroleum ether extracts as well as unsaponifiable fractions of both tested plants were similar .TLC extract of unsaponifiable matter showed two major spots with R_f values (0.64 and 0.42) for compound 1 and compound 2 respectively and the petroleum ether extract by fractionation on column chromatography gives two major spots with R_f values (0.22 and 0.72) for compound 3 and compound 4 their structure elucidation were confirmed by melting point and some spectral analyses (mass and H1-NMR spectrometry)

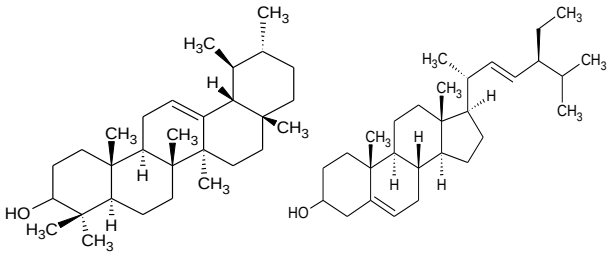
Compound 1 isolated as white solid amorphous of m.p. (184-186ºC) from unsaponifiable fraction R_f:0.64 (benzene:ethyl acetate - 8: 2 v/v) it gave a blue fluorescence under UV light and appeared as purble spot by spraying with sulfuric acid reagent (20% in ethanol) which proved terpenoid nature.**^{1}H-NMR** ($CDCl_3$, 500 MHz) of compound 1 revealed the presence of characteristic peaks an at δ 5.4 represented olefinic proton (1H, br*t* , *J*= 5.5, 6 Hz) at H-12, and a triplet proton shift at δ 3.5 correspond to H-3 (1H, br*t*,

J= 6.75, 7 Hz) in addition to presence of 8 methyl groups corresponding to H-23 - H-30 one doublet proton shifts at δ1.51(3H, d, *J*=3.1 assigned for H-30 and seven singlet protons for H-23 , H-24 . H-25 H-26, H-27, H-28 and H-29,). **EI-MS** (70 eV) showed [M]+ at m/z 426 corresponding to a molecular formula of $C_{30}H_{50}O$ with fragments at m/z 218 (base peak) 411, 393, 365, 203 ,189, 175 and 161 we can differentiated between and *α* and *β*-amyrin by examination of the relative intensities of the peaks at m/z189 and 203 peaks(Awad *et. al.,* 2018)(21), α-amyrin spectra shows both peaks with similar intensity, from 1H-NMR and EI-MS spectral data, by comparison with published data (Eseyin *et. al., 2018* **)(22)** ,compound 1 could be identified as pentacyclic triterpenes *α*-amyrin (3β-hydroxy-urs-12-en-3-ol)which isolated for the first time from *I.tricolor* species .

Compound 2 isolated as white needle crystals of m.p. (137-139ºC) from unsaponifiable fraction Rf: 0.42 (benzene: ethyl acetate - 8: 2 v/v) it gave a blue fluorescence under UV light and appeared as reddish violet spots by spraying with sulfuric acid reagent (20% in ethanol) which indicated steroidal nature. **^{1}H-NMR** of compound 2 revealed the presence of an olefinic proton at δ 5.34 (1H, t, *J* = 4.8 Hz) at H-6, and a multiplet proton shift at δ 3.52 correspond to H-3(1H, tdd, *J* = 4.5, 4.2) in addition to six methyl groups, two singlet proton shifts at δ 0.67 and 1.01 assigned for Me-18 and Me-19 respectively, three doublet proton shifts at δ 0.92, 0.81, 0.78 assigned for Me-21, Me-26 and Me-27 respectively and one triplet proton shift at δ0.82 assigned for Me-29. Mass: **EI-MS** (70 eV) showed [M]+ at m/z 414 corresponding to a molecular formula of $C_{29}H_{50}O$ with fragments at m/z 396, 381 329, 303, 273, 255, 231and 213. From 1H-NMR , EI-MS spectral data and by comparison with published data [**23**] ,compound 2 could be identified as *β*-Sitosterol (Stigmast-5-en-3-ol), which it was isolated from *I.fistulosa* before [23]. However, it is the first report of isolation of *β*-Sitosterol from *I.tricolor* species .

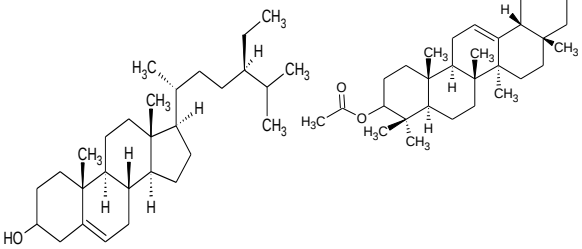
Compound 3 isolated as white powder of m.p. (169–170 °C) from fraction III R_f: 0.22 (benzene: ethyl acetate - 8: 2 v/v) it gave a blue fluorescence under UV light and appeared as purple spots by spraying with sulfuric acid reagent (20% in ethanol) which stated steroidal nature.**^{1}H-NMR** (CDCl3,500 MHz) displayed δ: 3.5 suggested a hydroxylated carbon atom C-3 (1H, CHOH), 5.73 suggested the presence of olefinic proton (1H, t, J = 4.8 Hz, H-6), signals at δ 5.02 and 5.15 intergrated to two protons suggested the presence of a double bond on the side chain (dd, J = 8.6 and15.2 Hz, H-22 and H-23),δ 0.87 to δ 1.1(m,18H, 6xCH3); at δ 1.1 to δ 1.20 (m,18 H, 9 x CH2),(8H, methine protons)appeared at δ 1.8 to δ 2.3. **EI-MS** (70 eV) showed [M]+ at m/z 412 corresponding to a molecular formula of C29H48O with fragments at m/z 394, 379, 255, 227 and 213 From 1H-NMR , EI-MS spectral data and by comparison with published data [24] compound 3 was found to be stigmasterol. it is the first report of isolation of stigmasterol from *I.tricolor* species .

Compound 4 isolated as white crystals, m.p. (227–228°C). from column fraction III R_f: 0.72 (benzene: ethyl acetate - 8: 2 v/v) it gave violet fluorescence under UV light and appeared as purple spot by spraying with sulfuric acid reagent (20% in ethanol)which significant for the terpenoidal nature. **^{1}H-NMR** (CDCl3, 500 MHz) showed the presence of eight methyl singlet signals resonating at 0.72 (3H, s, H-23), 0.97(3H, s, H-24), 0.93(3H, s, H-25), 0.95 (3H, s, H-26), 1.04 (3H, s, H- 27), 0.83(3H, s, H-28), 0.88(3H, s, H-29), 0.89(3H, s, H-30) ,the signals due to methylene and methine protons overlapped with each other and appeared as multiplet in the region of δ 1.0 – δ 1.92 integrating for 23 protons in addition characteristic signal at 2.05 (1H, s, OAc) indicated presence of acetate group, olefinic protons appeared 5.39 (1H, d , *J*= 4.4 Hz, H-12), deshielded signal of an oxygenated proton at 4.52 (1H, dd, J= 6.32, 10, H-3). Mass: **EI-MS** (70 eV) showed [M]+ at m/z 468 corresponding to a molecular formula for $C_{32}H_{52}O_2$ with fragments at m/z 408, 365, 271, 218,189 and 203 the intensities of the peaks at m/z189 and 203 are similar which indicated α-amyrin not *β*-amyrin [21]. From 1H-NMR , EI-MS spectral data and by comparison with published data[21] compound 4 was found to be: α-amyrin acetate. . it is the first report of isolation of from α-amyrin acetate *I.tricolor* species .



α-amyrin (1)

β-Sitosterol(2)



Stigmasterol(3)

α-amyrin acetate(4)

Figure 1. Triterpenoidal and steroidal compounds isolated and identified from *I.tricolor* , *α*-amyrin (1), *β*-sitosterol(2), stigmasterol(3) and *α*-amyrin acetate(4).

Bioloգical study

Antioxidant activity DPPH• free radical scavenging activity

The antioxidant activities for petroleum ether extracts of the *I.tricolor* and *I.fistulosa* were estimated by using DPPH• free radical scavenging and from the (Table:5) it can be noticed a significant increase in DPPH• inhibition activity of both pet. ether test extracts at 0.01 and 0.05 . While , at concentration of 0.1 ug /ml independent dose relationship was observed for *I.tricolor* , where insignificant change in percent of DPPH• inhibition was recorded compared to the concentration of inhibitor at 0.05ug/ml. However pet ether of *I.fistulosa* showed significant increase in DPPH• inhibition activity compared to vitamin C as standard reagent at concentrations of 0.05ug/ml and 0.1 ug/ml. our result not agree with [11] who stated that petroleum ether of *I. fistulosa* did not show any significant antioxidant activity in comparison with standard butylated hdroxy toluene (BHT).

Table 5: % Inhibition of petroleum ether extracts of *Ipomoea tricolor* and *Ipomoea fistulosa* using DPPH.

group	% of inhibition DPPH±SD		
	0.01 µg/ml	**0.05 µg/ml**	**0.1 µg/ml**
I.tricolor	45.93±0.22%	80.20±2.10	82.20±1.2
I.f istulosa	42.55± 0.8%	120±0.2%	125±0.5%
Vit c	85.90±3.20	89.90±1.20	90±0.7 %

Data are represented by mean ±SD of three replicate. Statistical analysis is carried out using two ways ANOVA coupled with CO-state computer program where similar letters are insignificant and different letters are significant at P≤ 0.05.

Anti-cholinesterase activity

the present study estimated for the first time the anticholinesterase activity of petroleum ether extract of *I.tricolor* and *I.fistulosa* leaves and the results showed insignificant change in inhibition percent of both tested extracts at concentration 10 ug /ml ,while at 20 ug/ml *I.tricolor* extract showed dose dependent relationship i.e. significant increase in percentage of inhibition of ACHE was recorded .Further, *I.tricolor* extract declared the highest percentages of inhibition in ACHE activity than *I.fistulosa* extract compared to dopenzile standard drug which showed dose dependent relationship.

Table 6: Cholinesterase % inhibition of petroleum ether extracts of *Ipomoea tricolor and Ipomoea fistulosa*.

Group	ACH esterase inhibition %	
	Conc 10 µg/ml	**20 µg/ml**
I.tricolor	25.250±1.20	29.300±1.20
I.fistulosa	23.120±1.30	25.200±1.00
Dopenzile	58 .60±2.77%	70.00±1.09%

Data are represented by mean ±SD of three replicate. Statistical analysis is carried out using two ways ANOVA coupled with CO-state computer program where similar letters are insignificant and different letters are significant at P≤ 0.05.

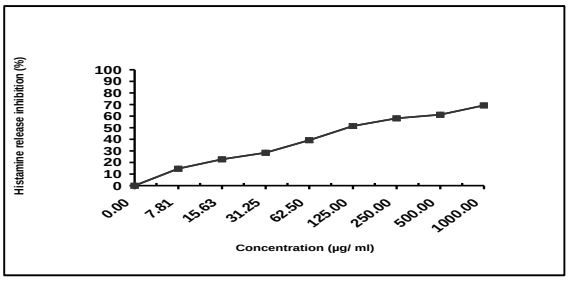
Anti-inflammatory activity

The anti-inflammatory activities of petroleum ether of *I.tricolor* and *I.fistulosa* were performed on U937 human monocytes (ATCC, Manassas, VA, USA) by using histamine release assay. the result in (Table 7) revealed that *I.tricolor* exhibited a significant higher activity with IC_{50} 117.4µg/ml than *I.fistulosa* with IC_{50} 341.49µg/ml in comparison with diclofenac (reference drug) with IC50 17.9µg/ml (figure 2,3,4).

Table :7 Histamine release inhibition % of petroleum ether extracts of *Ipomoea tricolor and Ipomoea fistulosa* .

Sample conc.	Histamine release inhibition %		
(µg/ ml)	***I.tricolor***	***I.fistulosa***	**Diclofenac**
1000	69.25±1.5	58.16±1.3	92.58
500	61.24±1.5	55.35±3.1	79.25
250	58.12±2.1	46.89±0.72	68.32
125	51.47±1.5	30.84±1.2	61.35
62.5	39.25±0.3	21.82±7.2	57.32
31.25	28.42±1.2	11.53±1.5	54.32
15.63	22.74±0.8	0	49.25
7.81	14.63±1.2	0	36.32
IC_{50} µg/ml	117.49	341.49	17.9

All determinations were carried out in triplicate manner and values are expressed as the mean ± SD. The IC50 value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions



Figure(2):Anti-inflammatory activity (histamine release inhibitory %) of different concentrations of *I.tricolor*

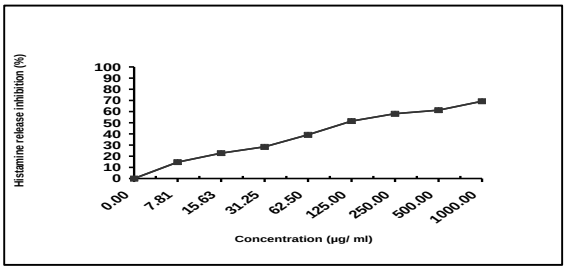
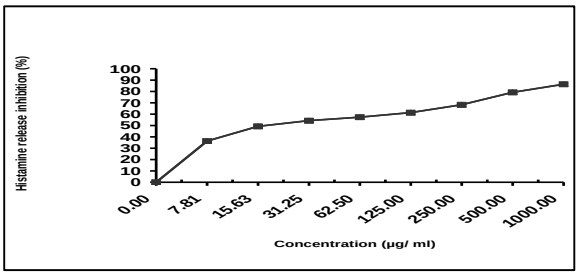


Figure (3): Anti-inflammatory activity (histamine release inhibitory %) of different concentrations of *I.fistulosa*.



Figure(4) Anti-inflammatory activity (histamine release inhibitory %) of different concentrations of reference.

Discussion

The biological activities of different *Ipomoea* species had been studied before as antioxidant, antidiabetic, anti-inflammatory and anticholinesterase , many researchers studied the *in vitro* antioxidant activities of *Ipomoea* species using different models of screening as free radicals are responsible for many diseases including diabetes mellitus ,ageing, cancer and arthritis Ambigal and Kumar [25, 26] related the antioxidant activity of *I. carnea* and *I. pes-caprae* alcoholic extracts respectively to the presence of polyphenols and flavonoids. In addition, ethanol extract of *I. batata* leaves demonstrated significant antioxidant activity [27].The ethyl acetate of *I.batata* had high scavenging activities towards DPPH• and higher ferric ion reducing antioxidant power (FRAP) related to the highest content of caffoyl quinic acid derivatives [28].Hasan[23] determined the cytotoxic, antioxidant and antimicrobial activities of *I. carnea*

Various cholinesterase inhibitors have been developed for the treatment of Alzheimer's disease (AD) which consider one of the most common forms of dementia ,causes a decline in cognitive function and language ability. These inhibitors including the naturally-derived plant source. [29] demonstrates that different extracts of leaves of *I. aquatica* had shown promising acetylcholinesterase inhibition activity with potent inhibition for hydroalcholic extract. Feitosa[30] estimated that methanol and ethyl acetate extracts of *I.asarifolia* as a potent acetylcholinesterase inhibition,

There is a great need for new safe products of natural source plant for treatment of inflammatory disorders as steroidal and non-steroidal anti-inflammatory drugs have many side effects ,the earlier studies reported that some species of *Ipomoea* possess anti-inflammatory activities in different mechanism of actions, the petroleum ether extract of *I.fistulosa* was previously reported before to have significant decrease in paw oedema in 24-hour observation and attributed the activity to the presence of *β*-sitosterol [9]. Khalid [31] stated that aqueous extract of *I. carnea* leaves possesses a significant anti-inflammatory activity as evidences in carrageenan induced paw oedema method. Pongrayoon [32] reported that *I. pes-caprae* has a significant anti-inflammatory activity, through the reduction of prostaglandin and leukotriene formation . Thus, in addition to earlier results, this work further supports the use of *I. tricolor and I. fistulosa* as an anti-inflammatory agents in traditional medicine.

The present study indicates that the pet.ether extracts of *I.tricolor* and *I.fistulosa* have antioxidant, anticholinesterase and anti-inflammatory activities which may be attributed to the presence of a good variety of bioactive compounds in both extracts, where they contain several saturated, unsaturated fatty acids, aliphatic and phenylated hydrocarbons, oxygenated sesqueterpenes , terpenoids as (*α*, *β*-amyrin), lupeol, simiarenol and steroid compounds as *β*-sitosterol and stigmasterol. These compounds were detected before from different *Ipomoea* species in addition to other plants and evaluated their biological activities as *β*-sitosterol isolated before from *I.fistulosa* by Hasan [23] and determined its antioxidant as well as its protective effects on serum and pancreatic tissue in strepotosotazin -induced diabetes[33]. The FAMEs obtained from vegetable oils (sunflower, soybean and

corn oils) mainly methyl linoleate in addition to methyl palmitate and methyl stearate had potent antioxidant and antifungal activities and indicates the potential of FAMEs as a source of antifungal and antioxidant activity [34]. The Pentacyclic lupane-type triterpenes (Lupeol) has been shown to exhibit various pharmacological activities included its beneficial activity against inflammation, cancer, arthritis, diabetes, heart diseases, renal toxicity and hepatic toxicity [35], α, β-amyrin, a pentacyclic triterpene ameliorates hyperglycemia and dyslipidemia, reduces atherogenic risk factor, and improves glucose tolerance in mice possibly by its anti-inflammatory and antioxidant effects[36],Thus, in addition to earlier results, this work further supports the use of *Ipomoea fistulosa and Ipomoea* tricolor as an anti-inflammatory agents in traditional medicine

Conclusion

The antioxidant ,anticholinesterase and anti-inflammatory activities of petroleum ether extracts of *I.tricolor* and *I.fistulosa* leaves may be due to the synergism between the total components identified of the extracts and mainly attributed to the presence of saturated, unsaturated hydrocarbons, FAME, sesqueterpens, triterpenes and sterols as major constituents as major constituents, we recommend to complete further studies on the two species which needed for incorporation them in different pharmaceutical preparations.

Acknowledgments

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Abbreviations

Ipomoea tricolor (*I.t*), *Ipomoea fistulosa* (*I.f*), unsaponifiable matter (USM), saponifiable matter(SM), fatty acids methyl ester (FAME), thin layer chromatography (TLC), nuclear magnetic resonance(NMR), electron ionization mass spectroscopy **(**EI-MS), 2-amino-2-methyl-1-propanol (AMP),2,2-diphenyl-1-picrylhydrazyl(DPPH), higher ferric ion reducing antioxidant power (FRAP), acetyl cholinesterase (AChE)**.**

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