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PHYTOCHEMICAL AND GC-MS STUDIES OF *CUCUMIS MELO* L SUBSP. AGRESTIS (NAUDIN) PANGALO FRUITS

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ABSTRACT

The objective of present study was to perform preliminary phytochemical screening, quantitative estimation of phytoconstituents, and gas chromatography mass spectroscopy studies of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruit. The petroleum ether, chloroform, alcohol and aqueous extracts of the dried fruits of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo were subjected to preliminary phytochemical screening, preliminary phytochemical screening of fruit extracts showed the presence of alkaloids, carbohydrates, glycosides, saponins, sterols, flavonoids, phenolic compounds, tannins, proteins and amino acids Quantitative estimation of alkaloids, carbohydrate, fat contents, saponin, total phenolics content and total flavonoids content in the fruit was done. Further, the hydroalcoholic extract was subjected to gas chromatography-mass spectroscopy (GC-MS) for identification of chemical constituents. GC-MS analysis of hydroalcoholic extract of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruits revealed the presence of 39 compounds. The results of this study indicates that extracts of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruits contains various phytoconstituents responsible for its medicinal properties

Keywords: *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruits, preliminary phytochemical screening, quantitative estimation, gas chromatography-mass spectroscopy (GC-MS)

INTRODUCTION

Cucumis melo L subsp. *Agrestis* (Naudin) Pangalo fruit belongs to Cucurbitaceae family which is herbaceous, generally climbing or trailing and usually found with tendrils. The family contains some of the earliest cultivated plants in old and new World tropics and subtropics(Petrus2014). The genus *Cucumis* is one of the economically most important genera of flowering plants and includes many commonly grown vegetables as well as ornamentals(Sutar *et al.,* 2013; Verma *et al.,* 2015) . *Cucumis melo*, the most variable species in *Cucumis*, originates from Africa (Renner *et al.,* 2007) and is at present cultivated all over the world. Species of *Cucumis* are characterized by a trailing, climbing, or bushy growth habit. It is native to dry areas of India being common throughout the South America and other parts of tropical Asia (Arora *et al.,* 2011; Chand *et al.,* 2012). *Cucumis melo* subsp. *Agrestis* is generally the only subspecies of *Cucumis melo* occurring naturally in Australia. Subsp. *melo* (containing Rock Melon and Honeydew Melon) being widely cultivated (De Wilde and Duyfjes 2007; Crase 2011). *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruits selected for the present study are widely available in Southern Haryana. The phytopharmacological evaluation proves medicinal importance(Karumi and Bobboi 1999). In this paper we are reporting the preliminary phytochemical screening and GC-MS analysis of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruits.

MATERIALS AND METHODS

Plant materials

Cucumis melo L subsp. *Agrestis* (Naudin) Pangalo fruits were collected in Southern region of Haryana, India in June 2015. The plant was taxonomically identified and authenticated by Dr. Anjula Pandey, Principal Scientist Raw Materials, Herbarium and Museum Division, NISCAIR, New Delhi, vide reference number NHCP/NBPGR/2016-15(*Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo) dated 17 March, 2016. A voucher specimen of the same has been retained in the Department for the future reference. The fruits were dried under shade and coarsely powdered for further study.

Evaluation Parameters

The evaluation of different parameter bitterness value, loss of drying, foaming index, crude fiber content, total ash, foreign organic matter, swelling index were studied (Anonymous 1996; WHO 2000; WHO. 2002)

Preparation of plant extracts

The powdered plant material (500g) was extracted with petroleum ether, chloroform, ethanol using soxlet apparatus. The extract obtained was concentrated by distilling off the solvent and recovering the same. The aqueous extract was prepared by using cold maceration method. The crude drug sample was macerated with distilled water for 24 hours and then filtered. The filterates were concentrated by rotary vacuum evaporator and lyophilized (Singh and Devi, 2018; Santhiya *et al.,* 2016; Singh and Devi, 2018). The extracts were kept in desiccator till further use. The percentage

Table 1: Different evaluation parameters of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruit

S.No	Parameters	*Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruit
1	Bitterness value	Bitter
2	Loss on drying (%)	2.6 ± 0.08 % w/w
3	Foaming index	Less than 100
4	Crude fiber content (%)	17.3
5	Total ash (%)	5.83 ± 0.14
6	Foreign organic matter (%)	1.83 ± 0.15 %w/w
7	Swelling Index	Absent

Table 2: Preliminary phytochemical screening of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruit extracts

S.No	Plant Constitutents	Test	Fruit Extracts			
			PE	C	M	A
1	Alkaloids	1. Mayer 'reagent	-	-	+	+
		2. Dragendroff' reagent	-	-	+	+
		3. Hager'reagents	-	-	+	+
2	Carbohydrates	1. Molish's reagent	-	-	+	+
		2. Fehling solution	-	-	+	+
		3. Benedict test	-	-	+	+
3	Flavonoids	1. Ammonia test	-	-	+	+
		2. Shinoda test	-	-	+	+
4	Glycosides	1. Baljet test	-	-	+	+
		2. Borntrager test	-	-	+	+
		3. Legal test	-	-	+	+
5	Saponins	1. Foam test	-	-	-	+
		2. Sodium bicarbonate	-	-	-	+
6	Sterols	1. Liebermann-Burchard test	-	+	+	+
		2. Salkowski reaction	-	+	+	+
7	Phenolics Compounds & tannins	1. Ferric chloride test	+	-	+	+
		2. Lead test	+	-	+	+
8	Proteins & Aminoacids	1. Biuret test	-	-	+	+
		2. Ninhydrin reagent	-	-	+	+

+ Present; -Absent; P-Petroleum ether; C- Chloroform; M- Methanol; A- Aqueous Extracts

Table 3: Test result of Carbohydrate, saponin, alkaloid and fat content of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo

S.No	Constitutents	Fruit methanol extract	Fruit aqueous extract	Fruit hydroalcoholic extract
1	Alkaloids (µg/ml)	4.32±0.06	5.34±0.03	12.56±0.05
2	Carbohydrate (%)	11.76±0.46	10.02±0.16	15.57±0.54
3	Fat contents (%)	13.6±0.5	7.56±0.45	12.7±0.07
4	Saponin (µg/ml)	7.42±0.05	09.11±0.14	16.56±0.04

yield of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruit petroleum ether, chloroform, methanol and aqueous extracts were 2.04% w/w, 1.78% w/w, 8.41% w/w and 9.94% w/w respectively. The extracts were further used to perform preliminary phytochemical screening.

Preparation of microwave assisted extract

The shade dried fruits of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo were crushed and coarsely grind. Then sample was kept in a four necked round bottom flask with solvent ethanol and extracted in U-Wave 1000 Microwave synthesis reactor (SINEO Microwave Chemistry Technology, China) at Power time mode. The instrument operates at an input power of 2000W with operating frequency of 2450MHz and works at atmospheric pressure. The real time temperature was monitored by high precision platinum resistance temperature sensor. The flask was connected to outside condenser through

Table 4: Total phenolic and flavonoid content of extracts of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruit

Extract	Total Phenolic content (mg/g)	Total Flavonoid content (mg/g)
PEF	4.2±0.01	6.5±0.07
CEF	3.9±0.04	7.1±0.08
MEF	98.62±0.13	76.97±0.05
AEF	79.2±0.27	80.35±0.78
HEF	113.03±0.74	97.68±0.82

PEF: petroleum ether extract fruit CEF: chloroform extract fruit, MEF: methanol extract fruit, AEF: aqueous extract fruit.; HEF: hydroalcholic extract fruit. Values are expressed as mean±SD (n=3).

Table 5: GC-MS spectral analysis of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruit extract

S. No	Retention Time	Name of Compound	Molecular Formula	Molecular Weight	Peak area %
1	5.109	4-Hydroxybutanoic acid	C4H8O3	104	0.18
2	5.531	1,2-Cyclopentanedione	C5H6O2	98	1.13
3	5.813	Itaconic anhydride	C5H4O3	112	2.35
4	6.194	2-Methyl-5-formylfuran	C6H6O2	110	0.56
5	6.615	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C6H8O4	144	1.18
6	8.284	Hyacinthin	C8H8O	120	0.21
7	8.419	Levulinic acid	C5H8O	116	0.53
8	9.466	4-Hydroxy-2,5-dimethyl-3(2h)-furanone	C6H8O	128	0.21
9	11.458	Pyranone	C6H8O4	144	8.05
10	12.446	Acetyl-1,2,3,4-tetrahydropyridine	C7H11NO	125	0.12
11	22.546	Fumaric acid, ethyl 2-methylallyl ester	C10H14O4	198	0.22
12	22.890	Ethyl N-(o-anisyl) formimidate	C10H13NO2	179	0.23
13	26.703	Tetradecanoic acid	C14H28O2	228	0.26
14	27.051	2-Pyrrolidin-1-yl-bicyclo[3.3.1] nonan-9-ol	C13H23NO	209	0.36
15	27.434	Pluchidiol	C13H2002	208	0.34
16	28.528	Diisobutyl phthalate; Palatinol IC	C16H22O4	278	0.16
17	29.481	Diisobutyl phthalate	C16H22O4	278	0.14
18	29.653	Phosphine, cyclohexyl[2-(2-pyridyl) ethyl]-	C13H20NP	221	0.31
19	29.820	Hexadecanoic acid, methyl ester	C17H34O2	270	0.73
20	30.915	Palmitic acid	C16H32O2	256	19.09
21	32.654	Fumaric acid, 2-octyl tridec-2-yn-1-yl ester	C25H42O4	406	0.22
22	33.013	Linoleic acid methyl ester	C19H34O2	294	3.32
23	33.134	Elaidic acid methyl ester	C19H36O2	296	1.07
24	33.628	Stearic acid Methyl ester	C19H38O2	298	0.33
25	34.234	Linoleic acid	C18H32O2	280	49.52
26	34.612	Octadecanoic acid	C18H36O2	284	2.94
27	35.324	10-Undecenoyl chloride	C22H40O	320	0.44

28	36.465	9-Tert-butyl-tricyclo[4.2.1.1 2,5] decane-9,10-diol	C14H24O2	224	1.25
29	36.764	Linolenic acid methyl ester	C19H32O2	292	0.62
30	37.242	Ethyl linoleate	C20H36O2	308	1.10
31	37.534	trans,trans-9,12-Octadecadienoic acid, propyl ester	C21H38O2	322	0.14
32	38.995	Ricinoleic acid methyl ester	C19H36O3	312	0.30
33	39.133	Cis-Decalindiol	C10H18O2	170	0.33
34	39.492	Ethyl Linoleate	C20H36O2	308	0.09
35	40.377	Bis(2-ethylhexyl) phthalate	C24H38O4	390	0.18
36	42.108	trans,trans-9,12-Octadecadienoic acid, propyl ester	C2H38O2	322	0.17
37	42.477	Linolein, 2-mono	C21H38O4	354	0.82
38	50.239	3-Hydroxy-12-ketocholanic acid	C24H38O4	390	0.28
39	52.019	Chondrillasterol	C29H48O	412	0.53

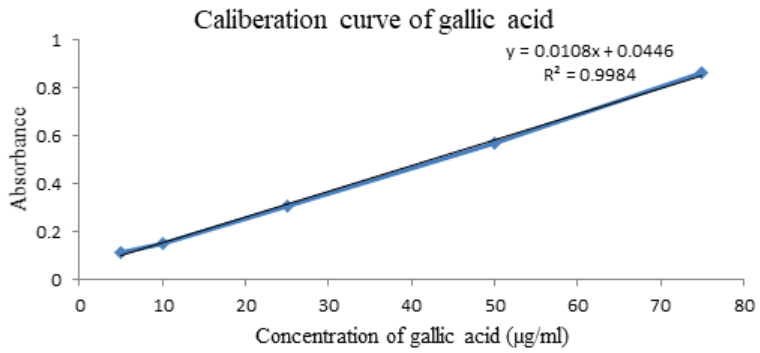

Figure 1: Calibration curve of gallic acid

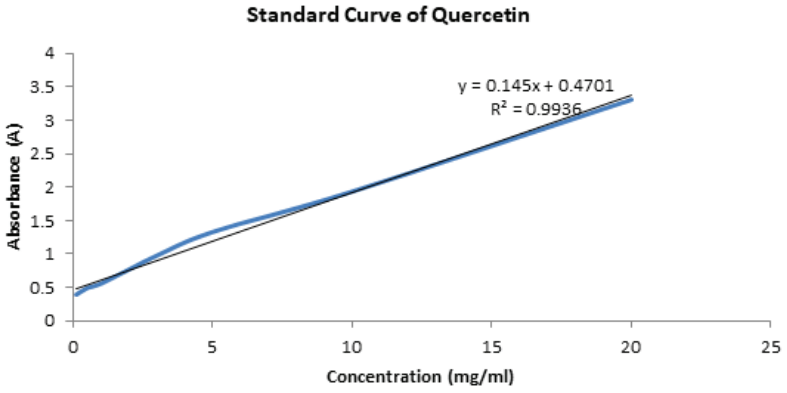

Figure 2: Calibration curve of quercetin

a glass connecting tube (19mm U) and a X shaped tube. The pulverized drug was extracted at different operating conditions (Microwave power, ethanol concentration (%) and different volume of solvent/g of drug) as suggested by experimental design. The extracts obtained by different techniques were cooled for 5 min before filtration. Further the extracts was filtered and concentrated under reduced pressure by a rotary evaporator at 60ºC. The experiment was conducted in triplicate and percentage yield (w/w) was determined. The extracts were kept in a desiccator before further analysis (Mittal and Nanda 2017; Singh and Devi 2018).

Preliminary phytochemical study

The petroleum ether, chloroform, methanol, aqueous and hydroalcholic extracts of fruits were subjected to preliminary phytochemical screening using standard method of analysis (Harborne 1973; Trease and Evans 1989; Otimenyin *et al.,* 2008; Kaur 2012. Singh *et al.,* 2012; Singh and Devi 2018). They were screened for the presence of alkaloids, carbohydrates, glycosides, saponins, phenolic and tannins, proteins and amino acids, sterols and flavonoids.

Quantitative estimation of phytoconstituents (Harborne 1973; Shabbir *et al.,* 2013; Singh and Devi 2018)

Total alkaloids

Each extract (50 mg) was mixed with 200 ml of acetic acid (10%) in ethanol; the beaker was covered and incubated for 4 h. The mixture was concentrated up to one third of its total volume. Ammonium hydroxide was added drop wise in the mixture until it formed precipitate. The precipitate was washed with ammonium hydroxide and then filtered. The filtrate (alkaloids) was calculated as percentage of the dried fraction.

Total carbohydrates

100mg of galactose were dissolved in 100 ml distilled water. Then 10 ml of strong galactose solution was dissolved in 100 ml distilled water to make the dilute galactose solution. Dilute galactose, mannose and sample were read(Clegg 1958).

Carbohydrates as galactose (or mannose) % = 25 x B/S x A

Where: B = reading of sample, A = reading of dilute galactose (or mannose) and S = weight of origin sample.

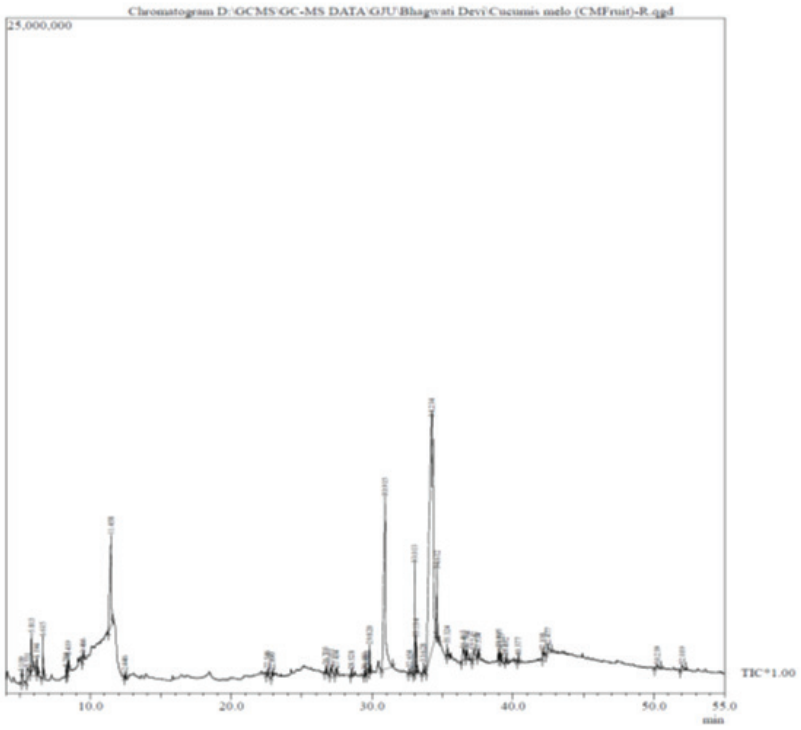

Figure 3: GC-MS Chromatogram of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo

Total saponin estimation

Methanol extract and each fraction (50 mg) were mixed in 100 ml of ethanol (20%). It was kept on heating for 4 h with continuous stirring at 55°C, than diluted with diethyl ether (20 ml) and washed with 5% sodium chloride. Saponins were estimated as percentage of the dried fraction(Harborne1973).

Determination of fat content

Accurately weighted an extraction flask containing a few glass beads and then added approximately 250 ml of petroleum ether in it. Extracted the sample (5g) contained in the thimble for at least 80 cycles in a minimum of 4 hours in a soxhlet extraction apparatus. Upon completion of the extraction, separated the unit and poured off the ether (and thimble) from the extractor into a large filter (to collect the thimbles) positioned on a container. Repeated until most of the ether was removed and the flask had very little ether left. Took apart the Soxhlet unit and placed flask on a steam bath to evaporate the remaining petroleum ether. Swirled flask initially to avoid boil-over. Dried flask and its contents in a mechanical convection oven at 100 - 102 °C for time required to obtain constant weight. Cooled to room temperature(Slinkard and Singleton 1977).

(%) Fat content = 100(B - C)/A

Where A = Sample weight; B = Weight of flask after extraction; C = Weight of flask prior to extraction

Total phenolic content

The total phenolic content was estimated by Folin-Ciocalteau reagent as earlier reported method. Gallic acid stock solution (1000µg/ml) was prepared. Various dilutions of standard gallic acid were prepared from this stock solution. Calibration curve (Figure 1) was plotted by mixing 1ml aliquots of 5, 10, 25, 50 and 75 µg/ml of gallic acid solutions with 5 ml of Folin-Ciocalteu reagent (diluted ten time) and 4 ml of sodium carbonate solution (75g/L). The absorbance was measured after 30 minutes at 765 nm(Rice-Evans *et al.,* 1995; and Singleton 1977). Total phenolic compound PC = Cg × V/M

Where; PC=total content of phenolic compounds in mg/g, in GAE (gallic acid equivalent); Cg= the concentration of Gallic acid established from the calibration curve in mg/mL; V=the volume of extract in mL; M=the weight of plant extract in gm. All the tests and analysis were run in triplicates and averaged.

The total flavonoid content

The total flavonoids content of various extracts was determined using a colorimetric method. A volume of 0.5ml of 2% aluminium chloride ethanol solution was added to 0.5mL of samples. After one hour at room temperature, the absorbance was measured at 420nm. A yellow color indicated the presence of flavonoids. Extract samples were evaluate at a final concentration of 0.1mg/ml (Rice-Evans *et al.,* 1995; Zhishen *et al.,* 1999; Oktay *et al.,* 2003;Malviya *et al.,* 2005). Total flavonoid content was calculated as quercetin equivalent (mg/g) using a standard curve with quercetin (0 - 100 µg/mL) as the standard and calculated using the formula: FC = Cq × V/M

Where; FC=total flavonoid content in mg/g, in quercetin equivalent; Cq = the concentration of quercetin established from the calibration curve in mg/mL; V=the volume of extract in mL; M=the weight of plant extract in g. All the tests and analysis were run in triplicates and averaged.

GC-MS analysis

The hydroalcoholic extract was directly used for the analysis. GC-MS analysis was carried out on a GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan) system with head space sampler (AOC-20s) and auto injector (AOC-20i), equipped with mass selective detector, having ion source temperature of 230°C, interface temperature of 270°C, a solvent cut time of 3.50 min, detector gain mode relative, threshold of 1,000 and mass range of 40 to 650 m/z. Compounds were separated using a Rtx 5 MS capillary column (Restek Company, Bellefonte, USA: crossbond 5% diphenyl/ 95% dimethyl polysiloxane) having dimensions 30 m (length) × 0.25 mm (diameter) × 0.25 µm (film thickness). The split mode was used at a ratio of 10:1. The temperature of the injector was initialized to 260°C, having a split injection mode, pressure 69.0 kPa. The temperature was programmed from 50°C (3 min), then further increased to 280°C at a rate of 10°C/min (24 min

hold). Helium (>99.999%) was used as the carrier gas at a linear flow velocity of 39.9 cm/s with constant flow of 1.21 mL/min and an injection volume of 1.0 µL was employed. The chemical constituents were identified by comparison of their retention indices (RI) relative to homologous alkane series (purchased from Sigma, St. Louis, USA) and by comparison of their mass spectral fragmentation patterns with those data provided in WILEY8.LIB, NIST08.LIB, NIST08s.LIB and NIST.LIB. Identification was assumed when a good match of mass spectrum and RI was achieved (Singh *et al.,* 2017; Singh and Devi 2018).

RESULTS AND DISCUSSION

Results of different parameters are shown in table 1.

Preliminary phytochemical study

The petroleum ether, chloroform, methanol and aqueous extracts of fruits were subjected to preliminary phytochemical screening using standard method of analysis. The results of tests for alkaloids,carbohydrates, glycosides, saponins, sterols, flavonoids, phenolic, tannins, proteins and amino acids are shown in table 2.

Quantitative estimation of phytoconstituents

Quantatitive estimation of alkaloids, carbohydrate, fat contents, saponin, total phenolics content and total flavonoids content was performed by using different extracts (table 3and table 4).

Total phenolic content

The total phenolic content was determined using Folin-Ciocalteu method. The results are reported as gallic acid equivalents by reference to standard curve ($y = 0.010x + 0.044$ and $r^2 = 0.998$, where y was the absorbance and x is concentration of gallic acid in µg/ml) as shown in figure 1. The results showed that the total phenolic content was more in hydroalcoholic extract than other extracts of fruit (table 4).

Total flavonoids content

The total flavonoids content of various extracts were calculated (mg/g) using the standard curve of quercetin from equation ($y = 0.140x+0.470$, $R^2 = 0.993$, where y was the absorbance and x is concentration of quercetin in µg/ml) as shown in figure 2. The total flavonoid content in various extracts are shown in table- 4**.** The total flavonoids content was found to be more in hydroalcholic extract followed by methanol, aqueous and chloroform extract(table 4) .

GC-MS analysis

GC-MS spectral analysis and chromatogram of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruit extract a showed presence of 39 phytoconstituents (table 5, figure 3).

The present research work was carried out to study the phytoconstituents of *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruit. The GC-MS investigation led to the identification of the presence of 39 phytoconstituents some of the bioactive compounds are 2,4-dihydroxy-2,5-dimethyl-3(2h)-furan-3-one, hyacinthin, linoleic acid, palmitic acid, pluchidiol, linoleic acid methyl ester, pyranone, chondrillasterol in the fruit .

CONCLUSION

The alcoholic, aqueous and hrdroalcoholic extracts showed presence of alkaloids, carbohydrates, glycosides, saponins, phenolic, tannins, sterols, flavonoids, proteins and amino acids. The results revealed the major compounds are fatty acid, esters and alkaloids. The presence of these phyto constituents reveals that *Cucumis melo* L subsp. *Agrestis* (Naudin) Pangalo fruit can further study for its different medicinal uses.

Conflict of interest – Nil

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