International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2022; 10(6): 22-27
Received: 13-10-2022
Accepted: 16-11-2022

A Aksharadevi
Department of Botany,
Bharathiar University,
Coimbatore, Tamil Nadu, India

P Sri Deepika
Department of Botany,
Bharathiar University,
Coimbatore, Tamil Nadu, India

Dr. K Chitra
Department of Botany,
Bharathiar University,
Coimbatore, Tamil Nadu, India

Corresponding Author:
Dr. K Chitra
Department of Botany,
Bharathiar University,
Coimbatore, Tamil Nadu, India

Phytochemical analysis and anticancer activity of *Majidea zanguebarica* Kirk Ex Oliv

A Aksharadevi, P Sri Deepika and Dr. K Chitra

DOI: https://doi.org/10.22271/flora.2022.v10.i6a.841

Abstract
Phytochemicals are secondary metabolites that are naturally produced by plants. The main aim of the study is to investigate the presence of phyto constituents in the flowers of *Majidea zanguebarica* through GC-MS analysis. Phytochemical screening was carried out using methanol, benzene, petroleum ether and chloroform extracts which revealed the presence of alkaloids, flavonoids, steroids, tannins, saponins, fatty acids, carbohydrates and cardio glycosides. GC-MS analysis of the methanolic flower extract evidenced that the presence of 28 compounds in the flowers of *M. zanguebarica*. The highest amount of carbohydrate and amino acid content were present in the methanolic flower extract 0.175 mg/g and 0.269 mg/g respectively. *In vitro* antioxidant activity such as DPPH radical scavenging activity, hydroxyl radical scavenging activity, total antioxidant activity, reducing power of the methanolic extract of flowers were analyzed. Radical scavenging activity was found to be higher in the methanolic extract of flowers. In MTT assay, IC_{50} value for methanolic flower extract found to be 25 µg/ml concentration. In DNA fragmentation study, the maximum DNA fragments was observed in the high concentration of methanolic flower extract. It was concluded that the methanolic flower extract has the tendency to activate the cell death process in the treated cells. This investigation might be further evaluated for its anticancer potential in a significant level.

Keywords: Phytochemical screening, GC-MS analysis, MTT assay, DNA fragmentation assay

1. Introduction
Majidea zanguebarica is a small tree belonging to Sapindaceae commonly known mgambo tree. The tree is native to East Africa and grows up to 5 meters (16ft) tall. The Sapindaceae family is a tropical and subtropical woody family represented by 150 genera and about 2000 species [1]d Sapindaceae family is known for its traditional medicinal uses as a diuretic, stimulant, expectorant, a natural surfactant, sedative, vermifuge, and against stomach ache and dermatitis in many parts of the world. Phytochemicals are naturally occurring, biologically active chemical compounds in plants [2]. It is believed that there may be about 4000 phytochemicals contained in plants that can be used to prevent, minimize, and remedial actions for strokes, cancer, or metabolic syndrome. The phytochemical analysis involves extraction, screening, and identification of bioactive compounds present in plant parts [3]. Many plants have been examined to identify new and effective anticancer compounds, as well as to elucidate the mechanism of cancer prevention and apoptosis. Chemical investigations of this family have led to the isolation of saponins, triterpenes, and flavonoids among other secondary metabolites [16]. Plants of this family occur as trees, herbs and lianas. Previous phytochemical investigations of these plants revealed the presence of fatty acids, cyan lipids, triterpenoids, saponins, polyphenols, flavonoids, Sphingolipids, alkaloids, coumarins, and Gallic acid derivatives. Their secondary metabolites exhibited interesting biological activities such as anti-plasmodial, anti-cancer, antiulcer, cytotoxic, antioxidant, and antibacterial [5].

2. Materials and Methods
2.1 Collection and identification of Plant
The flower material of *M. zanguebarica* Krik ex Oliv was collected from Bharathiar University campus, Coimbatore, during the month of March. The plant was identified by Botanical Survey of India, Southern Regional Centre, Coimbatore. (No. BSI/SRC/5/23/10-11/tech-117).

2.2 Cold maceration techniques
The dried plant materials (flowers) were extracted separately in maceration technique technique successively with different solvent (Petroleum ether, Chloroform, Benzene, Methanol).

The plant material was cold macerated technique with occasional stirring 72 hrs and was extracted. The solvent extracts were concentrated by rotary vacuum evaporator and then air dried. The dried extract obtained with each solvent was weighed. The percentage of yield was expressed in terms of air-dried weight of plant material [6].

2.3 Phytochemical Analysis of *M. zanguebarica*

flower extract of *using* different solvents to identify the major natural chemical groups such as alkaloids, Steroids, Cardiac glycosides, Anthraquinones, Carbohydrate, Terpenoids, Flavonoids, Saponins, Tannin, General reactions in this analysis reveal the presence or absence of these compounds in the flower extracts tested [7].

3. Results and Discussion

Table 1: Phyto chemical screening analysis of flower *M. zanguebarica*

Preliminary tests	Petroleum ether	Chloroform	Benzene	Methanol
Alkaloids	+	-	-	+
Steroids	+	+	+	+
Cardiac glycosides	+	-	+	+
Anthraquinones	-	+	+	+
Carbohydrate	+	+	+	+
Terpenoids	+	+	+	+
Flavonoids	+	-	+	+
Saponins	-	+	-	+
Tannin	+	+	+	+

+ Positive, - Negative.

The present study revealed that the presence of phytochemical constituents in solvents like petroleum ether, chloroform, benzene, methanol of flower *M. zanguebarica*. All the extracts of the flower showed different phytochemicals like tannins, flavonoids, phenolic compounds, saponins, terpenoids, glycosides. Positive and negative value indicated that the presence or absence of phytochemicals in preliminary tests (Table-2). The maximum number of secondary metabolites were observed in methanolic extract of flower. The phytochemical constituents known to exhibit medicinal as well as physiological activities [8].

3.1 Estimation of total carbohydrate

The estimation of carbohydrates was done in flower extract of *M. zanguebarica*. In this estimation the higher amount of carbohydrate was present in methanolic extract (0.175 mg /g) and the less amount of carbohydrate was present in chloroform extract (0.089 mg /g) (Fig:1).

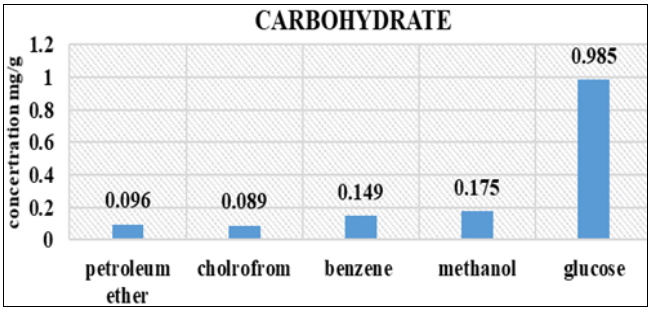

Fig 1: Estimation of total carbohydrate in flower extract of *M. zanguebarica*

3.2 Estimation of amino acid

The estimation of amino acid was done in flower extract of *M. zanguebarica*. In this estimation the higher amount of amino acid was present in methanolic extract of flower (0.269 mg /g) and the less amount of amino acid was present in petroleum ether extract of flower (0.188 mg/g). (Fig: 2)

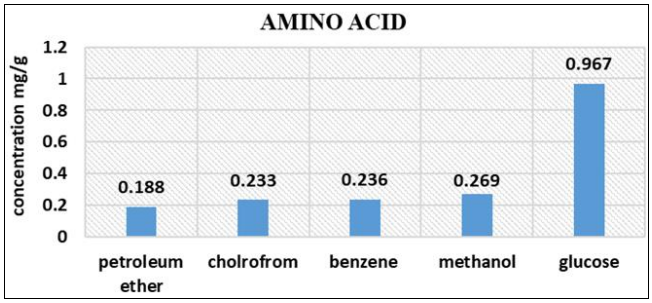

Fig 2: Estimation of total amino acid in flower extract of *M. zanguebarica*

3.3 Estimation of total phenols

High phenolic content was present in methanolic extract of flower, showed highest value of (0.857 mg/g) and petroleum ether extract showed lowest value of (0.334 mg/g). (fig:3) The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. [9]

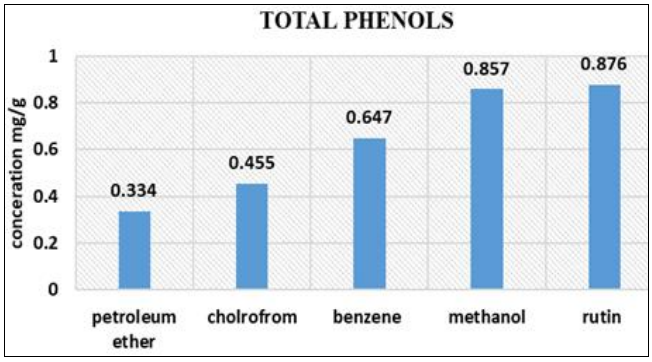

Fig 3: Estimation of total phenols in flower extract of *M. zanguebarica*

3.4 Estimation of total tannin

Total amount of tannin content present in the methanolic extract of flower showed highest value of (0.326 mg/g) and chloroform showed lowest value of (0.202 mg/g). (fig:4) Tannins bind to proline rich protein and interfere with protein synthesis [10].

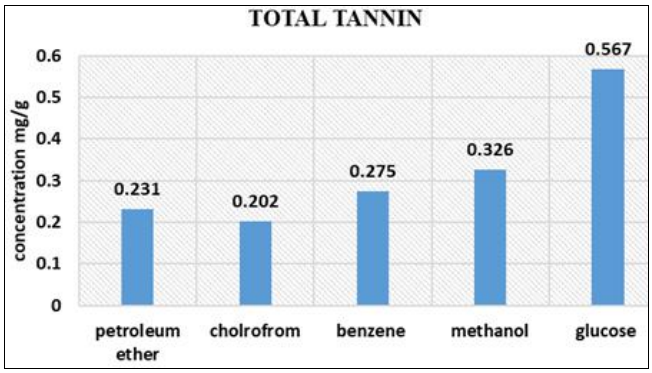

Fig 4: Estimation of total tannin in flower extract of *M. zanguebarica*

3.5 Estimation of total flavonoids.

Total amount of flavonoids content present in methanolic extract of flower, showed highest value of (0.922 mg/g) and

chloroform showed lowest value of (0.744 mg/g) (fig:5). Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances [11].

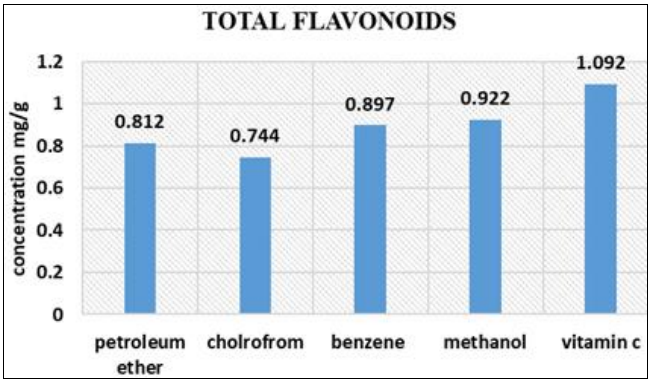

Fig 5: Estimation of total flavonoids in flower extract of *M. zanguebarica*

4. *In vitro* antioxidant assays

4.1 DPPH radical scavenging activity

Methanolic extract of flower showed maximum DPPH radical scavenging activity (0.345 mg/g) and petroleum ether showed minimum scavenging activity (0.234 mg/g). (Fig 6) The most important role of antioxidant is to suppress free radical mediate oxidation by inhibiting the production of free radicals through scavenging activity [12].

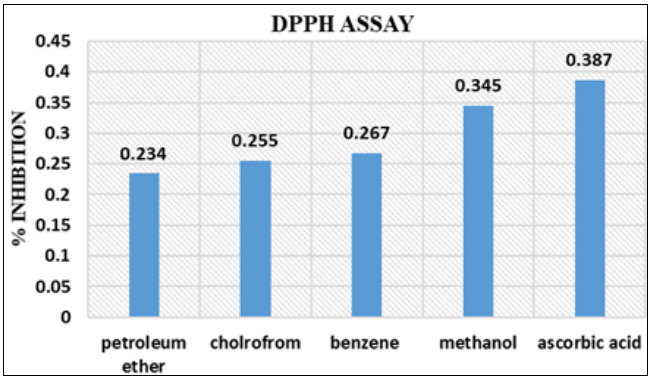

Fig 6: DPPH scavenging activity of flower extract *M. zanguebarica*

4.2 Hydroxyl radical scavenging activity

Methanolic extract of flower showed maximum Hydroxyl radical scavenging activity (0.922 mg/g) and petroleum ether showed minimum scavenging activity (0.534 mg/g). (Fig 7) [13].

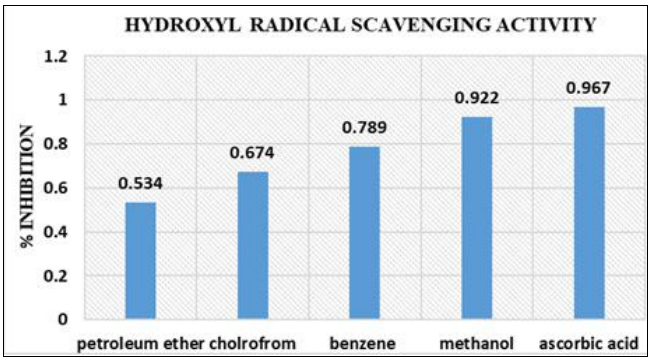

Fig 7: Hydroxyl radical scavenging activity of flower extract *M. zanguebarica*

4.3 Total antioxidant activity

Methanolic extract of flower showed maximum total antioxidant activity scavenging activity (0.537 mg/g) and petroleum ether showed minimum scavenging activity (0.311 mg/g). (Fig 8)

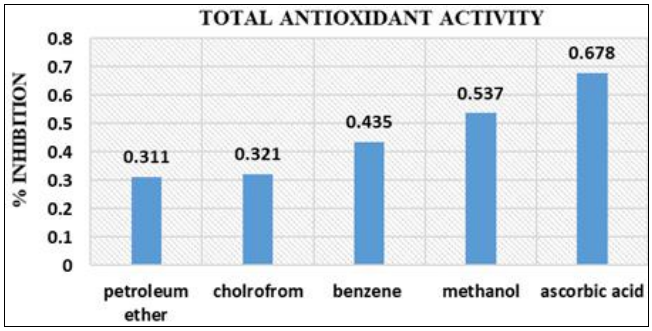

Fig 8: Total antioxidant activity of flower extract *M. zanguebarica*

4.4 Reducing power

Methanolic extract of flower showed maximum reducing power scavenging activity (1.977 mg/g) and petroleum ether showed minimum scavenging activity (0.875 mg/g). (Fig 9). The reducing power of the extracts may serve as a significant indicator of its potential antioxidant activity.

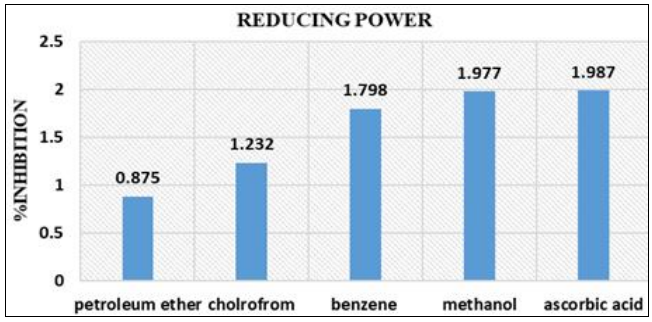

Fig 9: Reducing power of flower extract of *M. zanguebarica*

5. GC-MS analysis

The GC-MS analysis in methanol extract of *M. zanguebarica* Krik ex Oliv revealed that the presence of twenty eight compounds respectively Phytochemical constituents that could contribute the medicinal quality of the plant namely 3, 7, 11-Tridecatrinenenitrile, 4, 8, 12-trimethyl-, 1, 2, 3-Benzenetriol, Benzoic acid, 2-methoxy, Oxacyclotetradecan-2-one, Methyl 2-O-benzyl-d-arabinofuranoside, 1-Heptadecanamine, Benzocyclodecene, tetradecahydro, Salicylic acid, Ethanol, 2-(9-0ctadecenyloxy)-,(E)-, Benzoic acid, Glycerol 1-palmitate, 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl, 3-Octyne, 2, 2, 7-trimethyl-, Methyl 2-O-benzyl-d-arabinofuranoside, beta.-D-Glucopyranos, Acetic acid, 3-(1, 3) dioxolan-2-ylpropyl ester, 4H-Pyrrolo (3, 2, 1-ij) quinoline-4 -one, 1, 2, 5, 6-tetrahydro -6-methyl, 2, 4-Dihydroxy-2, 5-dimethyl-3 (2H) -furan-3-one, Methyl 2-O-benzyl-d-arabinofuranoside, 1, 2- Benzenedicarboxylic acid, bis (2-ethylhexyl) ester, Thymine, Verimol K, Maltol, Furane-2-carboxaldehyde, 5- (4-nitrophenoxymethyl)-, 1, 3-Dioxolane, 2-methyl-2- (4-methyl-3-methylenepentyl), 2-Butenoic acid, 2-methyl- and Methylparaben have antioxidant, antibacterial, antimicrobial, antimalarial, anti-inflammatory, antimycotoxigenic, analgesic, anti-proliferative, antiviral and anti-cancer activity. (Table: 2) (Fig: 10) [14].

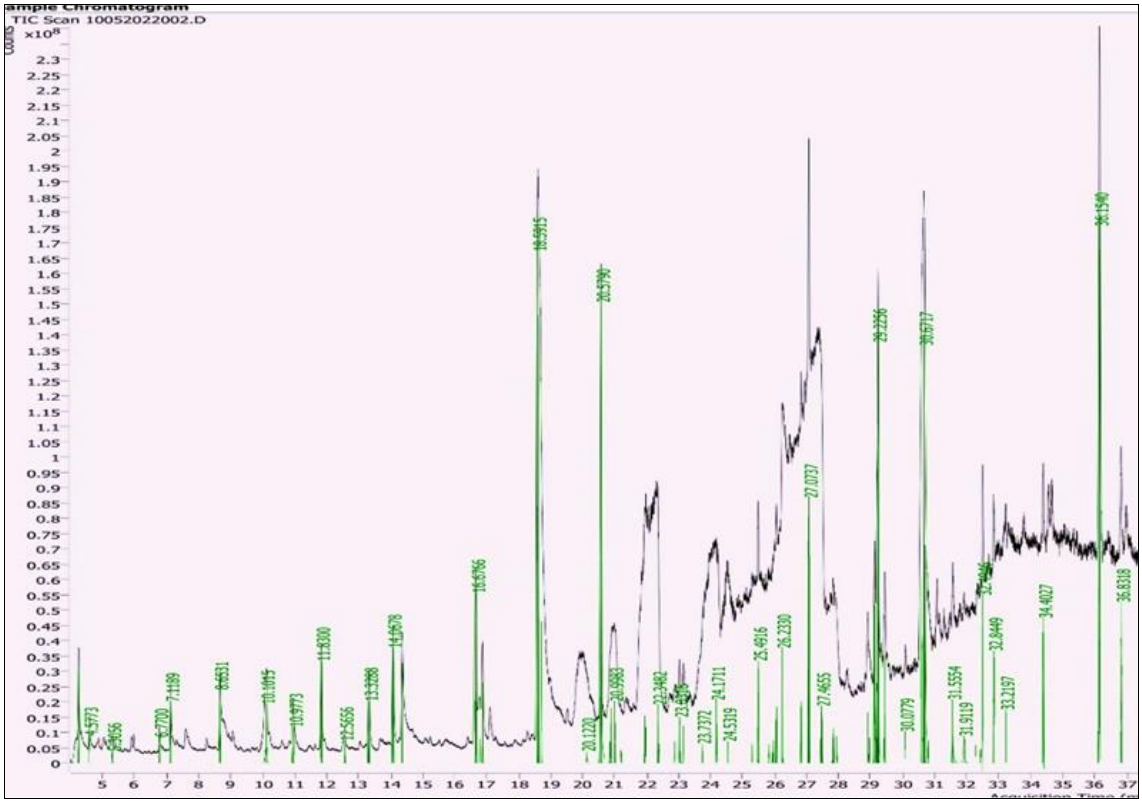

Fig 10: Chromatography of methanol extract in flower (*M. zanguebarica*)

Table 2: GC-MS analysis of Methanolic extract of flower (*M. zanguebarica*)

S. No	RT time	Name of the compound	Molecular formula	Molecular weight	Biological activity
	36.1540	3,7,11-Tridecatrinenenitrile,4,8,12-trimethyl-	$C_{17}H_{28}O_2$	264.4	Anti-cancer
	18.5915	1,2,3-Benzenetriol	$C_6H_6O_3$	126.1100	Diuretic, Antibacterial
	20.5790	Benzoic acid,2-methoxy	$C_8H_8O_3$	152.1473	Anti-inflammatory drug
	29.2256	Oxacyclotetradecan-2-one	$C_{15}H_{28}O_2$	240.38	Antimicrobial activity
	30.6717	Methyl2-O-benzyl-d-arabinofuranoside	$C_{13}H_{18}O_5$	254.28	Antiviral activity
	27.0737	1-Heptadecanamine	$C_{17}H_{57}N$	255.4824	Antimicrobial
	32.4946	Benzo cyclodecene, tetradecahydro	$C_{14}H_{26}$	194.36	Anti-proliferation
	16.6766	Salicylic acid	$C_7H_6O_3$	138.121	Antibacterial, analgesic, Antioxidant
	36.8318	Ethanol,2-(9-0ctadecenyloxy)-, (E)-	$C_{20}H_{40}O_2$	312.5	Anti mycotoxic genic activity, antibacterial
	34.4027	Benzocyclodecene, tetradecahydro	$C_{14}H_{26}$	194.36	Anti-proliferation
	26.2330	Benzoicacid,3,4,5-trihydroxy-, methyl ester	$C_8H_8O_5$	184.146	Antibacterial
	32.8449	Glycerol 1-palmitate	$C_{19}H_{55}O_4$	330.5026	Reductase inducing activity
	11.8300	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144.12	Anti-malarial, Anti-tuberculosis
	25.4916	3-Octyne,2,2,7-trimethyl	$C_{11}H_{20}$	152.28	Anti-cancer
	31.554	Methyl2-O-benzyl-d-arabinofuranoside	$C_{13}H^{18}O_5$	254.28	Anti-fungal, Anti-viral
	24.1711	1,2-Ethanediol,monoacetate	$C_4H_8O_3$	104.1045	Anti-microbial, Antioxidant, Anti-inflammatory
	20.9983	beta.-D-Glucopyranose,1,6-anhydro	$C_6H_{10}O_5$	162.14	Anti estrogen activity
	27.4655	Aceticacid,3-(1,3) dioxolan-2-yl Propylester	$C_8H_{14}O_4$	174.19	Antimicrobial
	23.0316	4H-Pyrrolo (3,2,1-ii) quinoline-4-one,1, 2, 5, 6-tetrahydro-6-methyl-	$C_6H_{10}O_5$	162.14	Antifungal, Antiviral
	7.1189	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	7.1189	Antioxidant
	10.1015	Thymine	$C_5H_6N_2O_2$	126.11	Anti-cancer, Antimicrobial
	30.0779	Verimol K	$C_{14}H_{12}O_4$	244.2427	Anti-inflammatory
	10.9773	Maltol	$C_6H_6O_3$	126.11	Anti-microbial
	6.7700	Furane-2-carboxaldehyde,5-(4-itrophenoxymethyl)-	$C_{11}H_7NO_4$	217.8	Anti-bacterial activity
	12.5656	Cyclobutane-1,1-dicarboxamide, N,N´-di-benzoyloxy	$C_{20}H_{18}N_2O_6$	382.4	Anti-microbial
	24.5319	1, 3-Dioxolane, 2-methyl-2- (4-methyl-3-methylenepentyl	$C_{11}H_{20}O_2$	184.27	Anti-inflammatory activity
	5.3056	2-Butenoic acid,2-methyl	$C_5H_8O_2$	100.12	Antimicrobial
	20.1220	Methyl paraben	$C_8H_8O_3$	152.15	Antibacterial

Among the identified bioactive compounds, 3, 7, 11-Tridecatrinenenitrile, 4, 8, 12-trimethyl- has highest percent peak area. This compound has antioxidant and anticancer activity.

6. *In vitro* anticancer activity

6.1 cell morphology analysis

The morphological changes of selected cancer cells in the absence and presence of extract at various concentrations was studied. It could be observed from that; control cells did not show any remarkable changes on their morphology. However, in the presence of extract the cells showed, improved cell shrinkage, membrane blebbing and forms floating cells in a dose-dependent manner. It is well accepted that cytological investigations elucidate the anti- proliferative effect routed through membrane blebbing, membrane instability and distressing the cytoskeleton of the cells by the methanolic extract of flower [15].

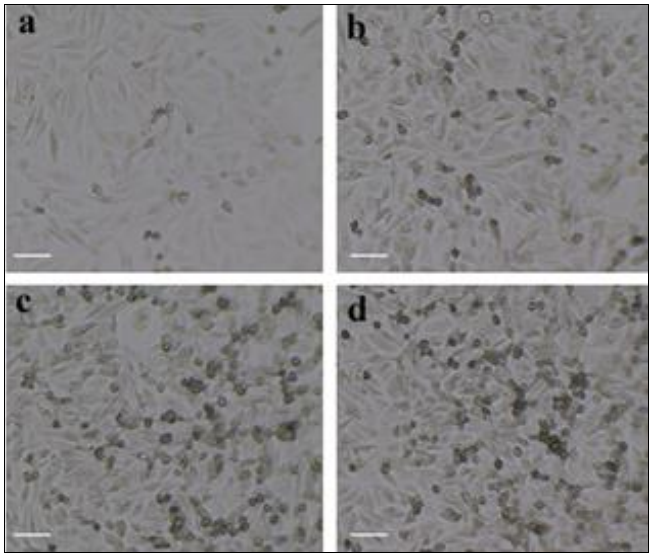

Fig 11: Cell morphology analysis of methanolic extract of flower *M. zanguebarica*

6.2 Cytotoxicity effect

The cytotoxicity effect of the methanolic flower extract was analyzed by using MTT assay. From the IC_{50} values, methanolic flower extract of different concentrations against cancer cells were tested, and it was found to be 19µg/ml in methanolic extract. The results showed that the observed IC_{50} values of the extract are low and significantly inhibited the proliferation of selected Human cervical cancer cells [15].

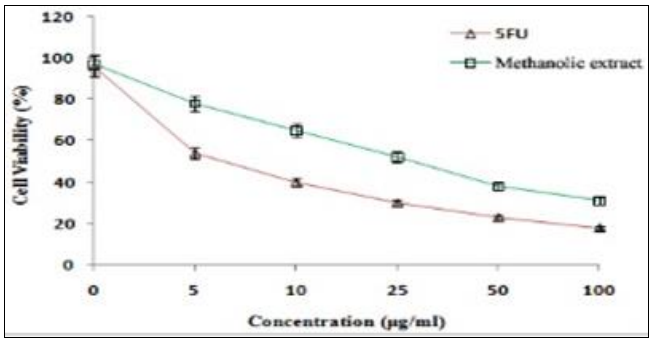

Fig 12: Cytotoxicity effect of methanolic extract of flower *M. zanguebarica*

6.3 DNA fragmentation

In order to assess the DNA fragmentation, DNA gel elution method was carried out and the results showed that the extract has significantly fragmented the DNA treated HeLa cells in a dose dependent manner. The different concentrated cell group DNA showed the fragmented pattern with response to the concentration of extract. Further it was noticed that, with the increase in the concentration of extract increases the fragmentation of DNA. Thus it is concluded that the extract has the tendency to activate the cell death process and it can be further evaluated for its anticancer potential [15].

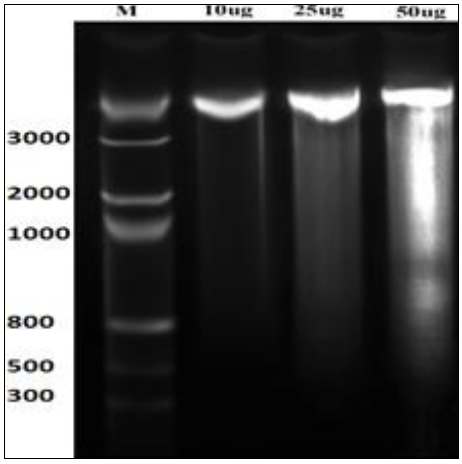

Fig 13: DNA fragmentation assay in methanolic extract of flower *M. zanguebarica*.

7. Conclusions

The result of this study showed the presence of phytochemicals such as alkaloids, steroids, tannins and cardiac glycosides in methanolic extracts of flower *M. zanguebarica*. Phenolic are the largest group of phytochemicals that account for most of the antioxidant activity in plants. Flavonoids are particularly beneficial, acting as antioxidants and giving protection against cardiovascular disease, certain forms of cancer. The GC-MS analysis of flower extract *M. zanguebarica* revealed the presence of bioactive compounds with important medicinal properties. Hence, the presence of these phytochemicals could be responsible for the therapeutic effects of the plants. The methanolic flower extract showed high cytotoxicity on cervical cancer cells. This cytotoxic effect caused inhibition in cell growth and the mechanism of this action was apoptosis in HeLa cells. Further investigation is required for possible development of novel drugs using some of the bioactive compound 3, 7, 11 -Tridecatrinenenitrile, 4, 8, 12 -trimethyl- found in flower of *M. zanguebarica*.

8. References

1. Abdulmajid A, Hamidon TS, Rahim AA, Hussin MH. Physicochemical studies of tamarind shell tannins as a potential green rust converter. Bio Resources. 2019;14(3):6863-6882.
2. Krishnaiah D, Devi T, Bono A, Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. Journal of medicinal plants research. 2009;3(2):067-072.
3. Kashyap CP, Arya V, Thakur N. Ethnomedicinal and phyto pharmacological potential of *Crataegus oxyacantha* Linn. –A review. Asian Pacific Journal of Tropical Biomedicine. 2012;2(2):S1194-S1199.
4. Shivakumara KN. Review on friedel-crafts acylation of benzene derivatives using various catalytic systems. Int. J Adv. Chem. Res. 2021;3(1):25-31. DOI: 10.33545/26646781.2021.v3.i1a.32
5. Sweetman C, Deluc LG, Cramer GR, Ford CM, Soole KL. Regulation of malate metabolism in grape berry and other developing fruits. Phytochemistry. 2009;70(11-12):1329-1344.
6. Bouabid K, Lamchouri F, Toufik H, Faouzi MEA. Phytochemical investigation, *in vitro* and *in vivo* antioxidant properties of aqueous and organic extracts of

toxic plant: *Atractylis gummifera* L. Journal of Ethno pharmacology. 2020;253:112640.
7. Sukalingam K. Preliminary phytochemical analysis and *in vitro* antioxidant properties of Malaysian 'Kundang' (*Bouea macrophylla* Griffith). Trends in Phytochemical Research. 2018;2(4):261-266.
8. Sofowra A. Medicinal Plants and traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria; c1993. p. 191-289.
9. Singh R, Singh SK, Arora S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. Fod Chem. Toxicol. 2007;45:1216-1223.
10. Han X, Shen T, Lou H. Dietry polyphenols and their biological significance. Int. J Mol. Sci; c2007. p. 950-988.
11. Brown JE, Rice-Evans CA. Luteolin rich artichoke extract protects low density lipoprotein from oxidation *in vitro*. Free Radical Res. 1998;29:247-255.
12. Krings U, Berger RG. Antioxidant activity of roasted foods. Food Chem. 2001;72:223-229.
13. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahuand A, *et al*. Indian medicinal herbs as source of antioxidants. Food Res. Int. 2008;41:1-15.
14. Marjorie C. Plant products as antimicrobial agents. Clincal Microbiol. Rev. 1996;12:564-582.
15. Murugan K, Dinesh D, Kavithaa K, Paulpandi M, Ponraj T, Alsalhi MS *et al*. (Hydrothermal synthesis of titanium dioxide nanoparticles: mosquitocidal potential and anticancer activity on human breast cancer cells MCF; c2016.
16. Rakariyatham K, Zhou D, Rakariyatham N, Shahidi F. Sapindaceae *(Dimocarpus longan and Nephelium lappaceum)* seed and peel by-products: Potential sources for phenolic compounds and use as functional ingredients in food and health applications. Journal of Functional Foods. 2020;67:103846.