Available online at **www.joac.info**

ISSN: 2278-1862

Journal of Applicable Chemistry

2024, 13 (4): 528-537

(International Peer Reviewed Journal)

Qualitative analysis of Phytochemicals Present in Aqueous Leaves Extract of *Eranthemum pulchellum*

Shyam Raj Yadav, Kumari Rinki, Manisha Kumari and Jai Prakash*

Department of Chemistry, S P Jain College, Veer Kunwar Singh University, Sasaram-821115, Bihar, **INDIA**

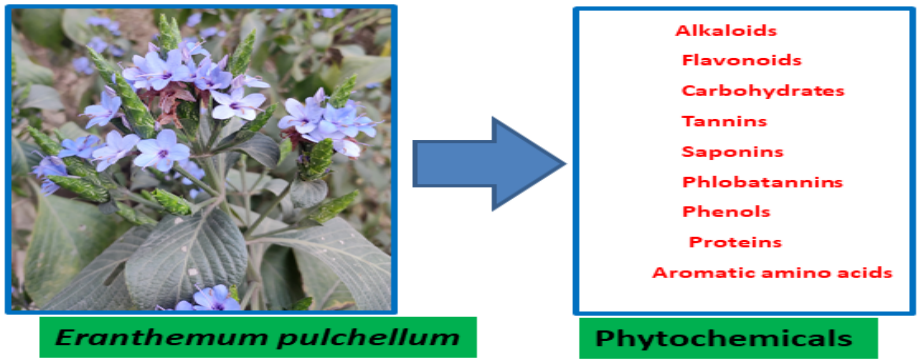
Email: jaibhu.patel@gmail.com

Accepted on 18th July, 2024

ABSTRACT

The chemicals obtained from plants are called phytochemicals. These phytochemicals are solely responsible for biological activities shown by a particular plant. Qualitative phytochemical analysis gives a preliminary idea about the biologically active compounds present in the plants. This screening leads to the detection of the bioactive entities present in plants which helps to find the lead compounds for development of new drugs. Present study deals the preliminary qualitative phytochemicals analysis of Eranthemum pulchellum leaves extract.

Graphical Abstract:



Phytochemicals present in leaves of *Eranthemum pulchellum*

Keywords: Phytochemicals, Bioactive, *Eranthemum pulchellum*, Nanoparticles

INTRODUCTION

The chemicals derived from plants are known as phytochemicals [1]. The prefix 'phyto' term originated from Greek, meaning 'plant'. They are produced via primary or secondary metabolism [2, 3]. Classification of phytochemicals based on their chemical structure has been shown in figure 1. They are usually bioactive compounds which play a key role in plant growth and development, regulating pollination and fertilization. They also provide defense against disease causing agents, hervivores, predators, and contenders [2]. Therefore, they have advantageous properties for producing

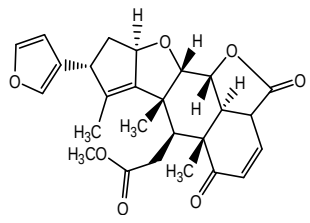
plants. However, intrinsic biological properties of phytochemicals may cause both adverse as well as beneficial effects on other organism. They have been used in traditional medicine without much of the knowledge of their mode of action. For example, salicin is a kind of phytochemical that reduces inflammation and pain. It was initially extracted from the bark of willow tree. Later, it was synthesized in labs and leads to become a very common precursor for over-the-counter drug, aspirin [4]. The roots of *Jatropha curcus* are used in India for the treatment of dysentery and diarrhea [5]. The ancient humans made poisonous arrows from the plant *Atropa belladonna* because this plant contains poisonous chemical, tropane alkaloids, but they did not have any structural knowledge [6].

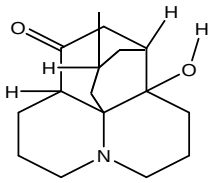
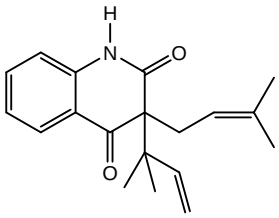
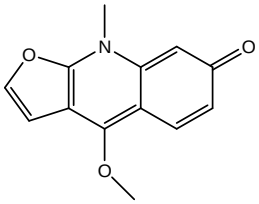
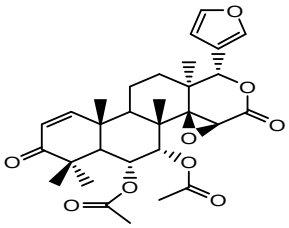
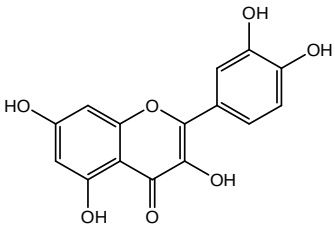
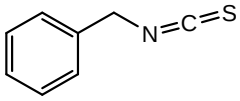
Phytochemicals with well explored roles in the body are known as essential nutrients which are required for normal physiological functions in humans [7]. Hsin-Chia Hung et al. studies suggest that increased fruit and vegetable consumptions are associated with a reduced risk of major chronic disease and especially in cardiovascular disease, but evidences are not enough in support of phytochemicals roles [8].

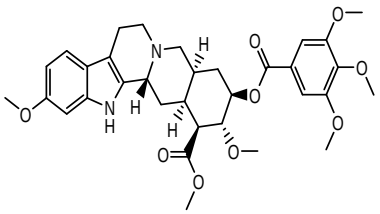
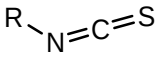
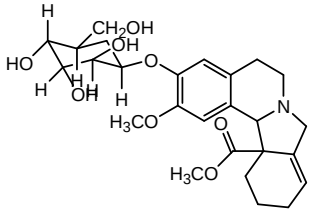
The epidemiological studies suggest that consumption of cruciferous vegetables is associated with a reduced risk of casualty of cancer at various sites, including the lung, rectum, breast, prostrate, and so on [9]. This cancer fighting activity is due to isothiocyanates bioactive compound present in cruciferous vegetables, and sulforaphane especially present in broccoli [9]. Flavonoids are naturally occurring phytochemicals that have polyphenolic structural nature. They are found in several fruits, vegetables, and beverages such as tea, apples, berries, onions, grape fruits, coffee, wine, soy, and chocolate. Several epidemiological studies suggest that flavonoids consumption lead to a lower risk of cardiovascular disease [10, 11]. Anthocyanin-rich strawberry fruit extracts were studied on B16-F10 murine melanoma cells [12]. It was found that they have great potential in reduction of cell proliferation, together with both the decreasing of the intracellular levels of polyamine, and the elevation of tissue transglutaminase activity [13]. Carotenoids such as lycopene and beta-carotenes help in neutralization of free radicals which are responsible to cell damage [14]. Unfortunately, a more research based studies are needed due to the lack scientific evidences about the roles of phytochemical compounds on humans. Therefore, phytochemical supplements are not recommended by health authorities or regulatory agencies. Currently, instead of lack of scientific evidences, eating a diet high in fruits and vegetables are recommended by many health authorities such as the American Cancer Society and the American Heart Association, so that an individual consumes an enough amount of phytochemical compound for long-term health benefits [15, 16]. Several phytochemicals have been further isolated and tested for various biological activities, among some are depicted in table 1.

In this present study, *Eranthemum pulchellum* was chosen as test plant. Further, Preliminary qualitative phytochemicals analysis was performed by using leaves extract of this plant.

Table 1. Phytochemicals and their biological activities

S. No.	Phytochemicals	Source plants	Applications	References
1	Nimbolide 	*Azadirachtaindica*	Anticancer activity against breast cancer and osteosarcoma	[17, 18]

	Isolycodolin			
2		*Melicopelunu-ankenda*	Antibacterial	[19]
	Buchapine			
3		*Euodiaroxburghiana*	Antiviral	[20]
	Melicarpinone			
4		*Melicopelunu-ankenda*	Antidiabetic and anti-inflammatory	[21]
	6α-acetoxygedunin			
5		*Carapaguianensis*	Antimalerial activity	[22]
	Quercetin			
6		*Psidiumguajava*	Antioxidant activity	[23]
	Benzyl isothiocyanate			
7		*Salvadorapersica*	Antibacterial	[24]

8	Reserpine 	*Rauwolfiaserpentina*	Anticancer activity	[25]
9	Isothiocyanates 	*Armoraciarusticana*	Antifungal and antibacterial activity	[26]
10	Hirsutine-3-O-glucopyranoside 	*Cocullushirsutus*	Antibacterial activity	[27]

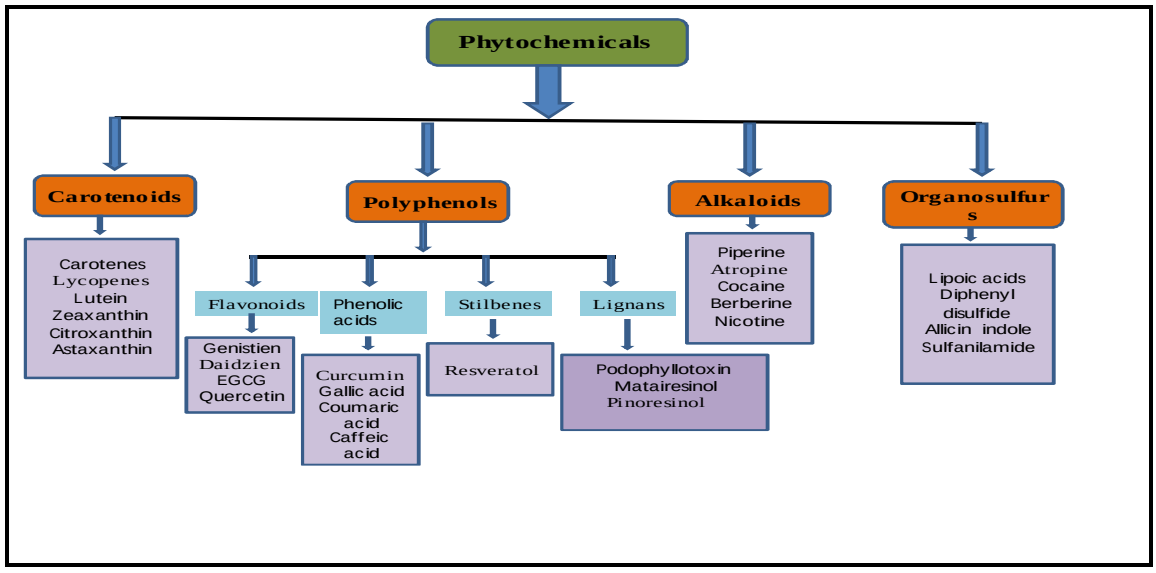


Figure 1. Classification of phytochemicals based on their chemical structure [12].

MATERIALS AND METHODS

This section illustrates the preparation of plant leaves extract and methods used for preliminary qualitative phytochemicals screening.

Preparation of plant leaves extract: The fresh 2 g of leaves of the *Eranthemum pulchellum* were washed thrice with double distilled water and chopped into small pieces. These chopped leaves were added in 25 mL of water and heated at 50°C for 30 min. Extract was cooled to room temperature and filtered using Whatman (grade -1) filter paper. Further, this filtered aqueous leaves extract was diluted to 50 mL and used for the preliminary qualitative phytochemicals analysis.

Preliminary phytochemicals screening: Preliminary qualitative screening for phytochemicals of test plant species was carried out with the following methods.

Test for alkaloids: 2 mL of the leaves extract was added to 2 mL of dilute hydrochloric acid. To this, 1 mL of Dragendroff's reagent was added. An orange precipitate was observed that indicated the presence of alkaloids [28]**.**

Test for flavonoids: A few drops of dilute sodium hydroxide were added to 2 mL of the leaves extract. Appearance of intense yellow color was observed, which became colorless on addition of a few drops of dilute hydrochloric acid indicated the presence of flavonoids [29].

Test for reducing sugar: 2 mL of the aqueous solution of the extract was added to 1 mL of each Fehling's A and Fehling's B solutions and heated for 2-5 min on water bath. The formation of brick red precipitate indicates the presence of reducing sugars [30].

Test for tannins: 2 mL of the aqueous solution of the extract were added to a few drops of 10 % ferric chloride solution. The appearance of green-blackish colour indicated the presence of tannins [31].

Test for saponins: 2 mL of aqueous extract was added to 6 mL of distilled water. The mixture was shaken vigorously. The persistence of foam was appeared that indicated the presence of saponins [1].

Test for phlobatannins: 2 mL of the aqueous solution of the extract was added into 2 mL dilute hydrochloride acid in a test tube. An appearance of red precipitate indicated the presence of phlobatannins [31].

Test for phenols: A few drops of 10 % ferric chloride solution were added to 2 mL of aqueous leaves extract. An appearance of blue or green color indicated presence of phenols [32].

Test for glycosides

Liebermann's test: 2.0 mL of acetic acid and 2 mL of chloroform with 4 mL of aqueous plant extract were heated on water bath. The mixture was allowed to cool at room temperature and added concentrated H_2SO_4 carefully. Appearance of no green color indicated the absence of aglycone, steroidal part of glycosides [33].

Keller-Kiliani test: 4.0 mL of glacial acetic acid with 1 drop of 2.0% $FeCl_3$ was mixed with the 5 mL aqueous plant extract and 1 mL of concentrated H_2SO_4. No brown ring between the layers indicated the absence of cardiac steroidal glycosides [33].

Salkowski's test: 2 mL of concentrated H_2SO_4 was added to the 5 mL of aqueous plant extract. No appearance of a reddish brown color indicated the abesence of steroidal aglycone part of the glycoside [33].

Test for proteins

Ninhydrin test: Few drops of ninhydrin reagent were poured into 2 mL of the aqueous plant extract in a test tube. Formation of deep blue colour indicated the presence of proteins [30].

Test for aromatic amino acids

Millon's test: A few drops of Millon's reagent were added to 2 mL of the aqueous plant extract in a test tube. Formation of white precipitate which on heating turned to brick red color indicated the presence of aromatic amino acids [34].

Test for carbohydrates

Molisch's test: a few drops of α-naphthol were added to 2 mL of the aqueous leaves extract in a test tube and shaken vigorously. To this mixture 1 mL of concentrated H_2SO_4 was then added slowly along the side of test tube and allowed to stand. The appearance of violet ring between layers indicated the presence of carbohydrates [35].

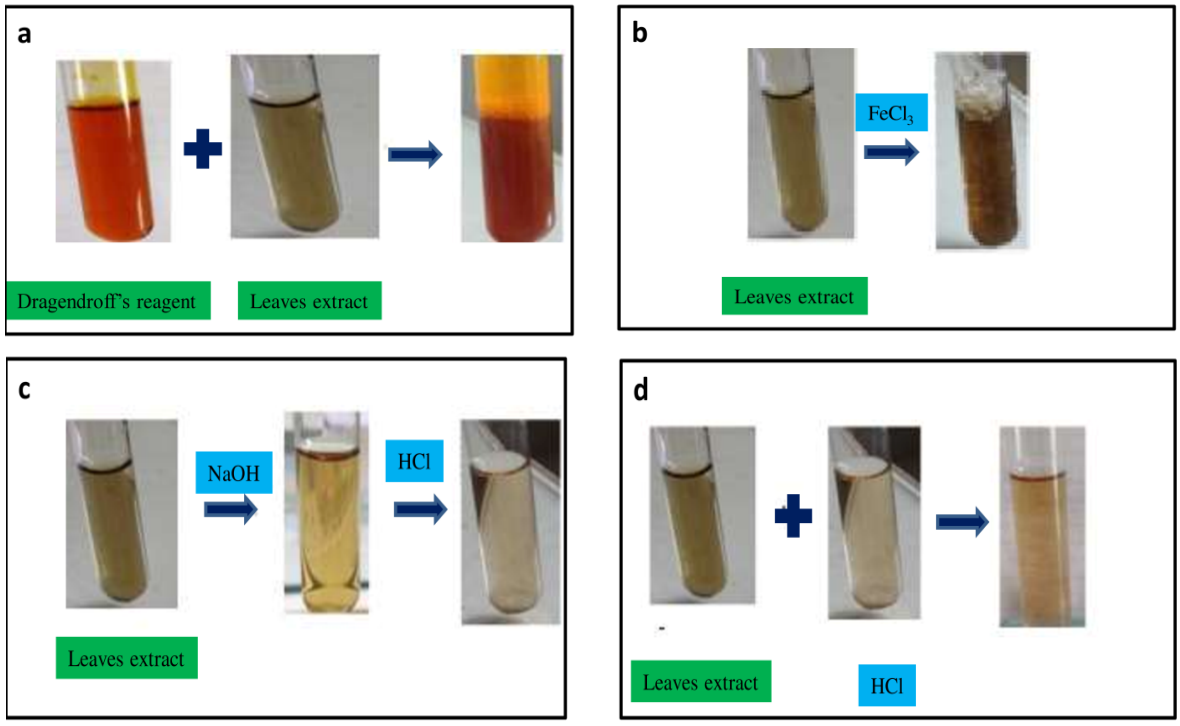
Test for steroids: 2 mL of the aqueous plant extract was poured into 5 mL of chloroform in a test tube. To this mixture 5 mL of concentrated H_2SO_4 was added via sides of the test tube. No color change observed indicated the absence of steroids [1].

Test for Terpenoids: 2 mL of chloroform and 2 mL of concentrated H_2SO_4 were added to 5 mL of the aqueous plant extract to form a layer in a test tube. No colour change was observed in reaction mixture indicated absence of the terpenoids [36].

RESULTS AND DISCUSSION

In the present study, preliminary qualitative phytochemical analysis has been performed by using different methods and aqueous leaves extract of *Eranthemum pulchellum* plant indicated the presence of phytochemical entities namely alkaloids, flavonoids, reducing sugar, tannins, saponins, phlobatannins, phenols, proteins, aromatic amino acids, carbohydrates, and absence of glycosides, steroids, and terpenoids. Obtained results are summarized in table 2 and figure 2.

The preliminary phytochemical screening tests may be advantageous in the initial identification of the biologically active compounds present in the test plant and this eventually may help to sketch the reaction mechanism for the synthesis of metal nanoparticles [37, 38]. This screening is also helpful in the detection of the bioactive entities present in medicinal plants and eventually may lead to development of new drugs [39]. Furthermore, this screening is also used to detect the bioactive entities that are poisonous to human's health [40].



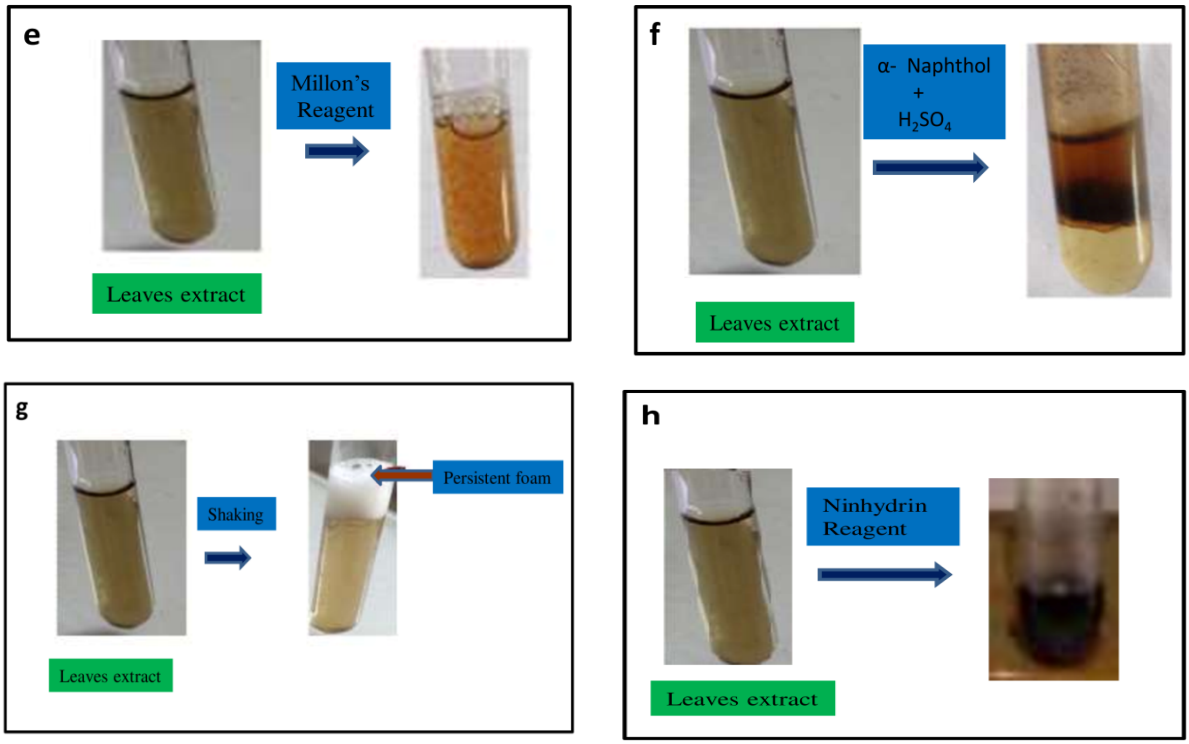


Figure. 2 Test for phytochemicals (a) Alkaloids (b) Tannins and phenols (c) Flavonoids (d) Phlobatannins (e) Aromatic amino acid (f) Carbohydrates (g) Saponins (h) Proteins.

Table 2. Phytochemical analysis of aqueous leaves extract of *Eranthemu pulchellum.*

S. No.	Phytochemical test	Result
1	Alkaloids	+
2	Flavonoids	+
3	Carbohydrates	+
4	Tannins	+
5	Saponins	+
6	Phlobatannins	+
7	Phenols	+
8	Glycosides	-
9	Proteins	+
10	Aromatic amino acids	+
11	Terpenoids	-
12	Steroids	-
13	Reducing sugar	+

+ present; - absent

APPLICATION

Phytochemical screening is the systematic process of analyzing crucial phytochemical entities present in plants and thus this work helps in identifying different classes of phytochemicals entities present in various parts of a plant responsible for providing a base to drug discovery. Moreover, the present study gives an important basis for further investigation into the separation and characterization of active phytochemical entities from the particular plants for the development of drugs. Thus the outcome of the research might be helpful in developing efficient drugs against numerous diseases.

CONCLUSION

The phytochemical screening analysis results indicated that the leaves extracts of *Eranthemum pulchellum* were composed of several potential bioactive entities that may be helpful in development of new drugs for the world. However, further a more research based investigations are needed to know the novel activities of phytochemicals present in the test plant.

REFERENCES

[1]. T. S. Bansode, B. K. Salalkar, Phytochemical analysis of some selected Indian medicinal plants, *Int J. Pharm Bio Sci.,* **2015**, 6, 1, 550 – 556.
[2]. R. J. Molyneux, S. T. Lee, D. R. Gardner, K. E. Panter, L. F. James, Phytochemicals: the good, the bad and the ugly, *Phytochemistry,* **2007**, 68, 2973–2985.
[3]. J. B. Harborne, H. Baxter, G. P. Moss, General Introduction. Phytochemical dictionary a handbook of bioactive compounds from plants, *London: Taylor & Francis,***1999**, 2nd ed..
[4]. D. R. Goldberg, Aspirin: Turn-of-the-century miracle drug. *Distillations,* **2009**. https://www.sciencehistory.org/distillations
[5]. E. A. Palombo, Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function, *Phytother. Res.,* **006**, 20, 717–724.
[6]. M. wink, Alkaloids: biochemistry, ecology, and medicinal applications. A short history of alkaloids,(Chapter 2),*Plenum Press, New York,***1998**.
[7]. K. Heneman, S. Zidenberg-Cherr, Nutrition and health info sheet phytochemical, *University of California, ANR Publication 8313,* **2008**.
[8]. H.C. Hung,K.J.Joshipura,R.Jiang,F.B.Hu, D.Hunter et al., Fruit and vegetable intake and risk of major chronic disease, *J.Natl. Cancer Inst.,* **2004**, 96, 21, 1577–1584.
[9]. N. Jugea, R. F. Mithena, M. Traka, Molecular basis for chemoprevention by sulforaphane: a comprehensive review, *Cell. Mol. Life Sci.,* **2007**, 64, 1105 – 1127.
[10]. S. M. Shenouda , J. S. Vita, Effects of flavonoid-containing beverages and EGCG on endothelial function, *J Am CollNutr.,***2007**, 26, 4,, 366S–372S.
[11]. M. G. Hertog, E. J. Feskens, P. C. Hollman, M. B. Katan, D. Kromhout, Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen Elderly Study, *Lancet,* **1993**, 342, 1007–1011.
[12]. S. K. Srivastava, S. Arora, S. Singh, A. P. Singh, Phytochemicals, microRNAs, and cancer: implications for cancer prevention and therapy, *Springer Science Business Media New York ,* **2013**. DOI 10.1007/978-1-4614-9326-6_9
[13]. C. Forni, R. Braglia, N. Mulinacci, A. Urbani, M. Ronci, Antineoplastic activity of strawberry (*Fragaria ananassa* Duch.) crude extracts on B16-F10 melanoma cells, *Mol. Bio. Syst.,* **2014**, 10, 1255.
[14]. A. T. Dinkova-Kostova, Phytochemicals as protectors against ultraviolet radiation: Versatility of effects and mechanisms, *Planta Med.,* **2008**, 74, 1548-1559.
[15]. J. J. Simon, Phytochemicals and cancer, *J. Chiropr Med.,* **2002**, 1, 3, 91–96.
[16]. B. V. Howard, D Kritchevsky, Phytochemicals and cardiovascular disease. A statement for healthcare professionals from the American Heart Association, *Circulation,* **1997**, 95, 11, 2591–2593.
[17]. P. Elumalai, R. Arunkumar, C. S. Benson, G. Sharmila, J. Arunakaran, Nimbolide inhibits IGF-I-mediated PI3K/Akt and MAPK signalling in human breast cancer cell lines (MCF-7 and MDA-MB-231), *Cell Biochem. Funct.,* **2014**, 32, 476–484.
[18]. J. F. Liu, C. H.Hou, F. L. Lin, Y. T. Tsao, S. M. Hou, Nimbolide induces ROS-regulated apoptosis and inhibits cell migration in osteosarcoma, *Int J Mol. Sci.,* **2015**, 16, 23405–23424.
[19]. E. M. Eliaser, J. H. Ho, N. M. Hashim, Y. Rukayadi, G. C. Lian, Phytochemical constituents and biological activities of *Melicopelunu-ankenda, Molecules,* **2018**, 23, 2708.

[20]. J. L. McCormick, T. C. McKee, J. H. Cardellina, M. R. Boyd, HIV inhibitory natural products. 26. Quinoline alkaloids from *Euodiaroxburghiana, J. Nat. Prod.,***1996**, 59, 469–471.
[21]. M. H. A. Zuaidy, A. A. Hamid, A. Ismail, S. Mohamed, A. F. A. Razis et al., Potent antidiabetic activity and metabolite profiling of *Melicopelunu-ankenda* leaves, *J. Food Sci.,* **2016**, 81, C1080–C1090.
[22]. T. B Pereira, L. F. R. Silva, R. C. N. Amorim, M. R. S. Melo, R. C. Z. Souza, In vitro and in vivo anti-malarial activity of limonoids isolated from the residual seed biomass from *Carapaguianensis* (andiroba) oil production, *Malaria Journal,* **2014**, 13, 317.
[23]. W. Nantitanon, S. Okonogi, Comparison of antioxidant activity of compounds isolated from guava leaves and a stability study of the most active compound, *Drug Discov. Ther.*, **2012**, 6, 1, 38–43.
[24]. A. Sofrata, E. M Santangelo, M. Azeem, A. K. Borg-Karlson, A. Gustafsson et al., Benzyl isothiocyanate, a major component from the roots of *Salvadorapersica* is highly active against Gram-negative bacteria, *PLoS One,* **2011**, 6, 8, e23045.
[25]. S. A. Abdelfatah, T. Efferth, Cytotoxicity of the indole alkaloid reserpine from *Rauwolfia serpentina* against drug-resistant tumor cells, *Phytomedicine*, **2015**, 22, 2, 308–318.
[26]. H. W. Park, K. D. Choi, I. S. Shin, Antimicrobial activity of isothiocyanates (ITCs) extracted from horseradish (*Armora ciarusticana*) root against oral microorganisms, *Biocontrol Sci.,***2013**, 18, ,3, 163–168.
[27]. A. K. Misra, P. Gouda, Phamacological study of alkaloid hirsutine-3-oglycopyranoside isolated from roots of *Cocullushirsutus*, *Intl J Pharmacogn Phytochem Res.,* **2014**, 6, 2, 317–319.
[28]. S. K. P. Nair, K. Ganesan, M. Sinaga, N. Letha، S. B.Gani, Preliminary phytochemical screening of different solvent extracts of leaves of *Echeveria elegans*rose, an endangered Mexican succulent herb, *Journal of Global Biosciences,* **2015**, 5, 3429–3432.
[29]. P. Shah, H. A. Modi, M. D. Shukla and S. K. Lahiri, Preliminary phytochemical analysis and antibacterial activity of *Ganodermalucidum* collected from dang district of Gujarat, India, *Int. J. Curr. Microbiol. App. Sci.,* **2014**, 3, 3, 246–255.
[30]. M. Boxi, Y. Rajesh, K. V. Raja, B. Praveen, K. Mangamma,. Extraction, phytochemical screening and in-vitro evaluation of anti-oxidant properties of *Commicarpuschinesis* (aqueous leaf extract). *Int. J. Pharm. Biosci*,. **2010**, **1**, 537–547.
[31]. M. S. Auwal, S. Saka, I. A. Mairiga, K. A. Sanda, A. Shuaibu et al., Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica*(Thorn mimosa), *Veterinary Research Forum,* **2014**, 5, 2, 95 – 100.
[32]. R. Roghini, K. Vijayalakshmi, Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of *Citrus paradise, IJPSR ,* **2018**, 9, 11, 4859–4864.
[33]. R. Gul, S. U. Jan, S. Faridullah, S. Sherani, N. Jahan, Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to balochistan, *Scientific World Journal,* **2017**, 1–7. https://doi.org/10.1155/2017/5873648
[34]. A. Kumar, K. K. Jha, D. Kumar, A. Agrawal, A. Gupta, Preliminary phytochemical analysis of leaf and bark (mixture) extract of *Ficusinf ectoria* plant, *The Pharma Innovation,* **2012**, 1, 71–76.
[35]. A. H. Lanjwani, I. H. Ghanghro, A. B. Ghanghro, T. M. J. Khuhawar, M. J. Channa, Qualitative examination of phytochemicals from some indiginous medicinal plants. *Sindh Univ. Res. Jour. (Sci. Ser.),* **2015**, 47, 2, 261–264.
[36]. R. Narasimhan ,Sathiyavani, Phytochemical screening and evaluation of protein content in the seed extracts of *Cucurbita maxima ,Int. J. of Pharm. Life Sci.,***2014**, 5, 7, 3637–3642.
[37]. A. Usmani, P. P. Dash, A. Mishra, Metallic nanoformulations: Green synthetic approach for advanced drug delivery, *Mater SciAdv Compos Mater,* **2018**, 2, 1–4.
[38]. D. K. Verma, S. H. Hasan, R. M. Banik, Photo-catalyzed and phyto-mediated rapid green synthesis of silver nanoparticles using herbal extract of *Salviniamolesta* and its antimicrobial efficacy. *J PhotochemPhotobiol B,* **2016**, 155, 51–59.

[39]. A. M. Karande, H. V. Kamble, V. H. Kumbhar, S. R. Kane, C. S. Magdum, Preliminary phytochemical screening of *Glochidionellipticum*,*Euro. J. Exp. Bio.,* **2016**, 6, 4, 41–45.
[40]. Q. Fardiyah, Suprapto, F. Kurniawan, T. Ersam , A. Slamet, Preliminary Phytochemical Screening and Fluorescence Characterization of Several Medicinal Plants Extract from East Java Indonesia, *IOP Conf. Ser.: Mater. Sci. Eng.*, **2020**, 833, 012008. doi:10.1088/1757-899X/833/1/012008