*Journal of Cell and Tissue Research Vol. 17(1) 6035-6041 (2017)*
*(Available online at www. Tcrjournals.com)*
***ISSN: 0973-0028; E-ISSN: 0974-0910***

**Original Article**

# PHYTOCHEMICAL AND GC-MS STUDIES ON TRADITIONAL HERBACEOUS PLANT PUMPKIN (*CUCURBITA PEPO*) LEAF

**ARUL PRIYA, R. AND SARAVANAN, K.**

Post Graduate and Research Department of Zoology, Nehru Memorial College (Autonomous), Puthanampatti 621 007, Tiruchirappalli, Southern India.
E. mail: kaliyaperumalsaravanan72@gmail.com, Cell: 09443757052

Received: February 2, 2017; Accepted: February 25, 2017

***Abstract: To analyze the presence of phytochemical compounds from the leaves of Cucurbita pepo (Pumpkin) by preliminary phytochemical screening and GC-MS studies. The dried powder leaves of C. pepo were extracted successfully by Soxhlet using ethylacetate and hexane solvents. All the prepared extracts were used to preliminary phytochemical screening. GC-MS analysis was performed to identify the phytocompounds present in the ethylacetate and hexane extracts of C. pepo leaves. Phytochemical analysis of extracts showed the presence of major classes of phytochemicals. GC-MS results revealed the presence of 7 phytoconstituents in ethylacetate extract and 4 phytoconstituents in hexane extract. Among these, α-amyrin, phytol and morin are medically important. Results of this study may provide a foundation for designing new drug for several diseases.***

**Key words:** Phytochemical screening, GC-MS analysis, *Cucurbita pepo.*

## INTRODUCTION

Plants are known to contain innumerable biologically active compounds [1], which possess antibacterial, antidiabetic, anticancer, antioxidant activities [2]. One hundred and nineteen secondary plant metabolites derived from plants are used as drugs globally [3]. Therefore, it is imperative and of utmost significance to carry out a screening of these plants in order to validate their use in folk medicine and reveal the active principle by isolation and characterization of their constituents. Cucurbita pepo is an herbaceous plant, belonging to a gourd family, Cucurbitaceae. The plant is good source of nutrients such as vitamin A and C. In many parts of the world, *C. pepo* has been used to treat tapeworm infection, hypertrophy of the prostate, urinary problems, and burns [4,5]. Moreover, it also has various health benefits such as antioxidant, antimicrobial, anti-inflammatory, cancer preventive, anti-diabetic and anti-hypertensive effects [6,7]. However, bioactive compounds present in this plant are yet to be identified. Hence, the present study was designed to investigate for the presence of various phytochemicals in the leaf of *C. pepo* which evokes various therapeutic effects.

## MATERIALS AND METHODS

**Collection of Plant material:** The leaves of *C. pepo* were collected from Kondichettipatti village (Latitude 11°21' N and Longitude 78°15' E), Namakkal District, Tamilnadu. The leaves were washed thoroughly with tap water to remove dust and then dried under the shade at room temperature. The dried leaves were ground using kitchen blender to obtain course powder.

**Preparation of extracts:** The leaf powder of *C. pepo* was extracted by Soxhlet extraction using ethylacetate and hexane solvents for 48 hrs each. The extracts were dried by hot air oven.

**Phytochemical screening of *C. pepo* leaf extract:** The ethylacetate and hexane extracts of *C. pepo* leaves were used for qualitative screening of phytochemicals such as alkaloids, carbohydrates, flavanoids, glycosides, cardiac glycosides, proteins, saponins, steroids, tannins and terpenoids by standard biochemical procedures [8,9].

**GC-MS analysis:** For the identification of bioactive compounds in the extracts, it was subjected to GC-MS analysis. It was performed using a JEOL GC MATE II instrument with the following conditions: Front inert temperature 220°C; Column HP 5Ms; Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1ml/min. The oven temperature was 50 to 250°C @ 10°C/min. The ion chamber temperature and GC interface temperature was 250°C. The Quadruple Double Focusing Analyzer was used for mass analysis: Photon Multiplier tube was used for detection. Mass spectra were taken at 70eV. All data were obtained by collecting the full-scan mass spectra within the scan range 50-600 amu. The composition of the crude extract constituents was expressed as a percentage by peak area.

**Identification of compounds:** The bioactive compounds in the ethylacetate and hexane extracts of *C. pepo* leaves were identified based on the GC retention time. Interpretation of mass spectrum of GC-MS was done by comparing with the data base at National Institute Standard and Technology (NIST). The name of the compound, molecular weight, molecular formula and structure of the compounds of the plant extracts were also retrieved from NIST, Guidechem, Chemspider and Pubchem Libraries.

## RESULTS

**Preliminary phytochemical screening:** The extracts of *C. pepo* leaf were screened for the qualitative determination of secondary metabolites. Results of the phytochemical analysis showed the presence of major classes of secondary metabolites such as steroids, saponin, protein, cardiac glycosides, and terpenoids in ethylacetate extract; and steroids, flavonoids, saponin, cardiac glycosides, glycosides, and terpenoids in hexane extract (Table 1).

**Chemical composition of extracts by GC-MS Analysis:** The ethylacetate extract showed seven peaks in the GC-MS chromatogram (Table 2 and Figs. 1,2) which were identified according to their retention time. They were 4H-1Benzopyran-4-one,7-hydroxy-2-[4-methoxyphenyl]-,4H-1-Benzopyran-4-one,3,5,7-trihydroxy-2-[4-hydroxyl-3-methoxyphenyl], 4H-1Benzopyran-4-one,2-[3-chloro-2-hydroxyphenyl]-5-hydroxy-3,7,8-trimethoxy-, α-amyrin, dodecanoic acid, 1,1'-biphenyl-4-ylcarbonylmethyl ester, 4H-1benzopyran-4-one, 5,7-dihydroxy-2-[2-methoxyphenyl] - and flavone, 2',3,5,7-tetramethoxy-. The hexane extract showed four peaks in the chromatogram which were identified based on their GC retention time. They were phytol, tritriacontane, α-amyrin and morin (Table 2 and Figs. 3,4).

## DISCUSSION

Plants contain huge diversity of photochemical compounds, many of them are reported to be biologically active compounds and are responsible for exhibiting wide range of pharmacological activities. The bioactive secondary metabolites have been shown to reduce the risk and progression of diseases such as cancer, cardiovascular, neurodegenerative diseases, etc by scavenging free radicals through various biological mechanisms [10]. Several studies are available on phenolic compounds have reported their usefulness in exhibiting potential biological activities such as antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, antimicrobial, anticancer etc. [11 ]. Many plants contain non-toxic glycosides that can be hydrolyzed to give phenolic compounds which are toxic to microbial pathogens [12]. Flavonoids and tannins are considered to be the most promising polyphenolic compounds among plant secondary metabolites [13]. Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, antidiabetic, antioxidant, anti-inflammatory as well as antitumor activities [14]. Tannins are a heterogeneous group of polyphenolic compounds. Plant extracts with tannins are used as anti duodenal tumours, and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals [15].

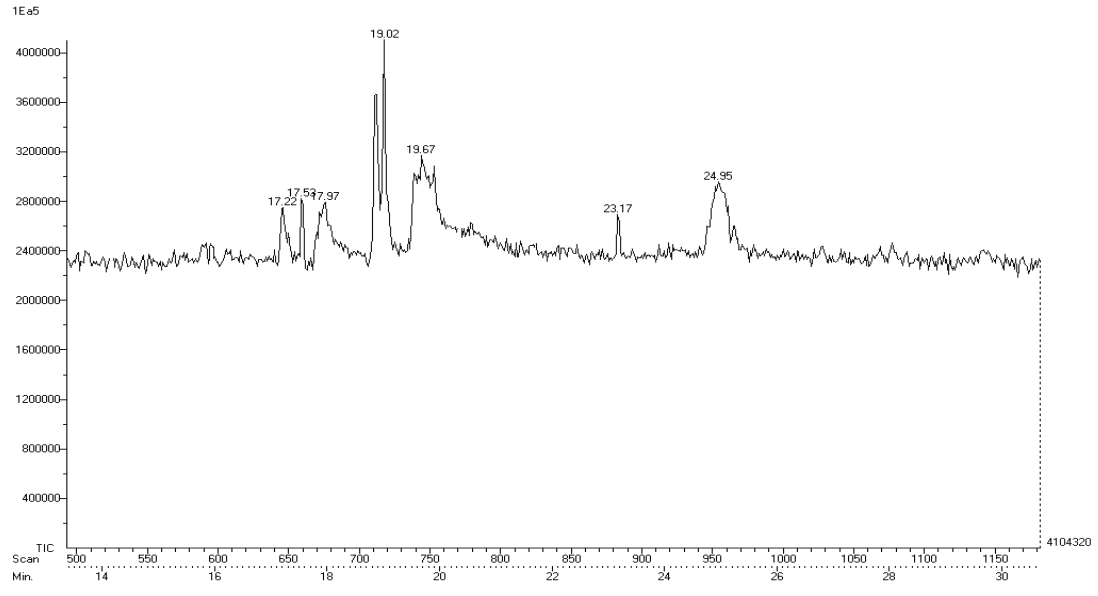
**Table 1:** Results of preliminary phytochemical screening of ethanolic and hexane extract of *C. pepo* leaves. (+ = Present - = Absent)

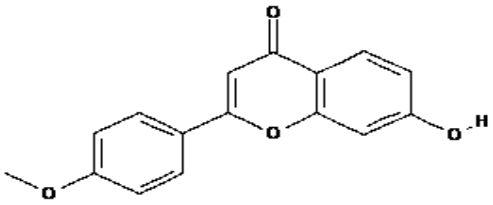
| Phytochemical test | Name of the test | Ethylacetate extract | Hexane extract |
|---|---|---|---|
| Tannins | Lead acetate test, $FeCl_3$ test | - | - |
| Steroids | Liberman Burchards test | + | + |
| Flavanoids | Alkaline reagent test, Con $H_2SO_4$ test, $FeCl_3$ test | - | + |
| Saponins | Emulsion test, Foam test | + | + |
| Proteins | Xanthoproteic test | + | - |
| Alkaloids | Hager's test, Mayer's test, Wagner's test | - | - |
| Carbohydrates | Barfoed's test, Benedicts test, Fehling's test | - | - |
| Glycosides | Bromine water test, Fehling's test | - | + |
| Cardiac glycosides | Con $H_2SO_4$ test | + | + |
| Terpenoids | Liberman Burchards test, Con $H_2SO_4$ test | + | + |

**Table 2:** Chemical composition of ethyl acetate and hexane extracts of *C. pepo* leaf.

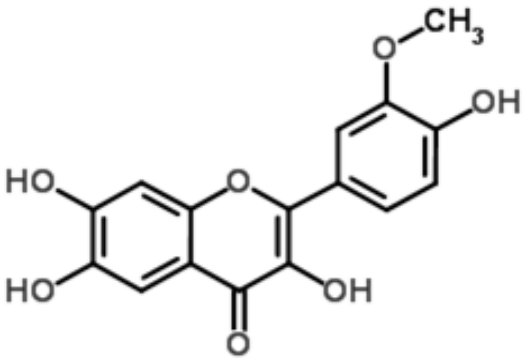
| Extracts Name | S. No | Compound Name | Retention Time (min) | Molecular Weight (d) | Molecular Formula | Peak Area (%) |
|---|---|---|---|---|---|---|
| Ethyla-cetate Extract | 1 | 4H-1Benzopyran-4-one, 7-hydroxy-2-[4-methoxyphenyl]- | 17.22 | 268 | $C_{16}H_{12}O_4$ | 12.97 |
| | 2 | 4H-1-Benzopyran-4-one, 3,5,7 -trihydroxy-2-[4- hydroxyl-3-methoxyphenyl] | 17.98 | 316 | $C_{16}H_{12}O_7$ | 12.68 |
| | 3 | 4H-1Benzopyran-4-one, 2-[3-chloro-2-hydroxyphenyl]-5-hydroxy-3,7,8-trimethoxy- | 19.67 | - | $C_{18}H_{15}ClO_7$ | 14.97 |
| | 4 | α- Amyrin | 24.95 | 427 | $C_{30}H_{50}O$ | 13.96 |
| | 5 | Dodecanoic acid, 1,1'-biphenyl-4-ylcarbonylmethyl ester | 23.17 | 395 | $C_{26}H_{34}$ | 12.70 |
| | 6 | 4H-1Benzopyran-4-one, 5,7-dihydroxy-2-[2-methoxyphenyl- | 17.53 | - | - | 13.34 |
| | 7 | Flavone, 2',3,5,7-tetramethoxy- | 19.02 | 342 | $C_{19}H_{18}O_6$ | 19.38 |
| Hexane extract | 1 | Phytol | 19.05 | 297 | $C_{20}H_{40}O$ | 26.90 |
| | 2 | Tritriacontane | 25.88 | 465 | $C_{33}H_{68}$ | 25.02 |
| | 3 | α – Amyrin | 24.97 | 427 | $C_{30}H_{50}O$ | 26.81 |
| | 4 | Morin | 20.4 | 302 | $C_{15}H_{10}O_7$ | 21.28 |

**Fig. 1:** GC-MS chromatogram of ethyl acetate extract of *C. pepo* leaves

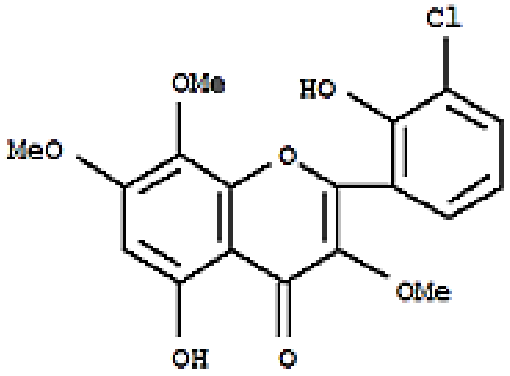




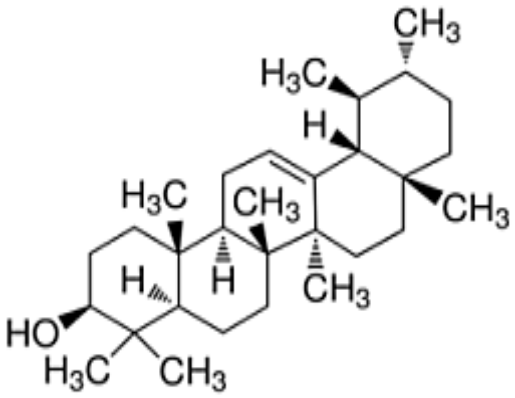
4H-1Benzopyran-4-one, 7-hydroxy-2-[4-methoxyphenyl]-



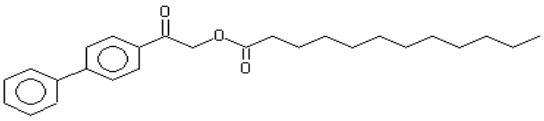
4H-1-Benzopyran-4-one, 3,5,7 -trihydroxy-2-[4- hydroxyl-3-methoxyphenyl]-



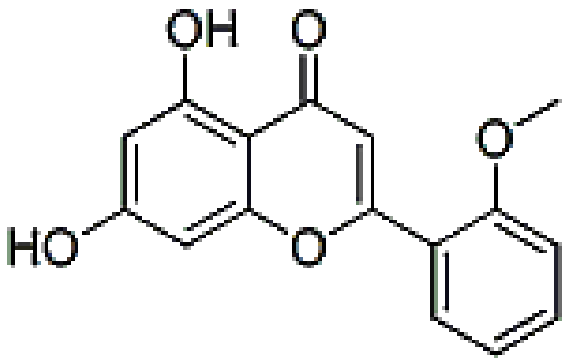
4H-1Benzopyran-4-one,2-[3-chloro-2-hydroxy-phenyl]-5- hydroxy-3,7,8-trimethoxy-



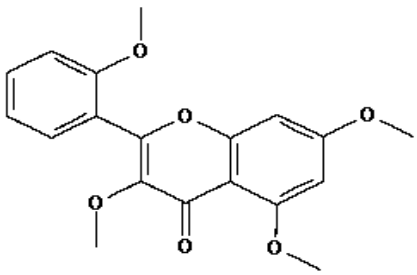
α-Amyrin



Dodecanoic acid, 1,1'-biphenyl-4-ylcarbonylmethyl ester



4H-1Benzopyran-4-one, 5,7-dihydroxy-2-[2-methoxyphenyl-



Flavone, 2',3,5,7-tetramethoxy-

**Fig. 2:** Chemical structure of bioactive compounds of ethyl acetate extract of *C. pepo* leaves.

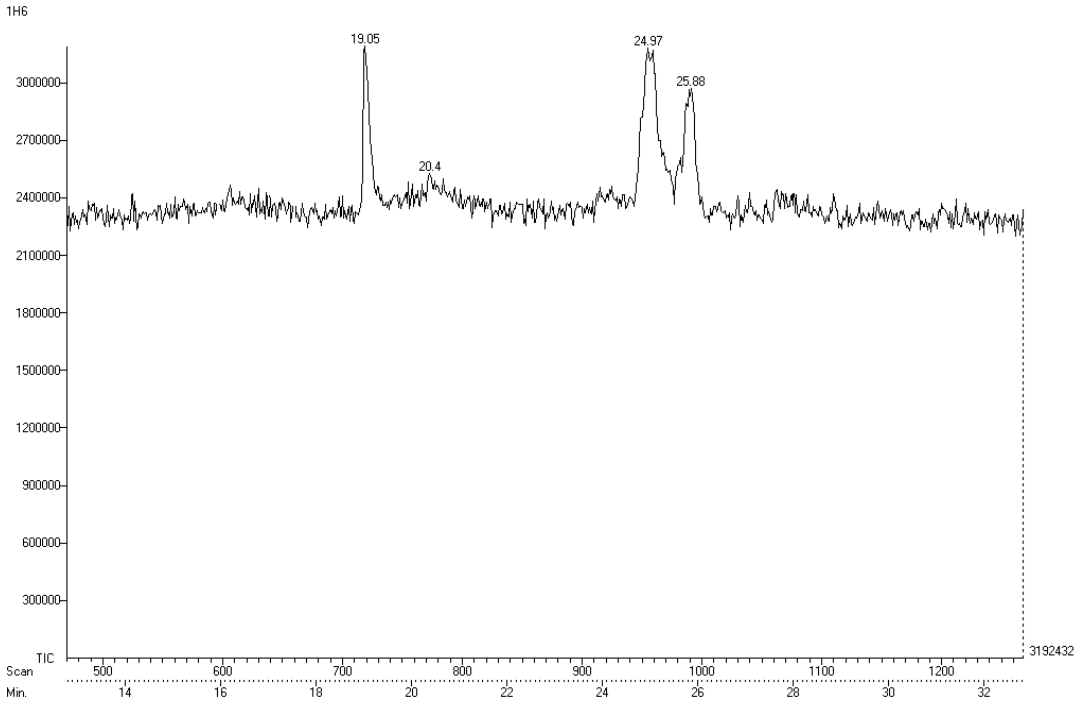
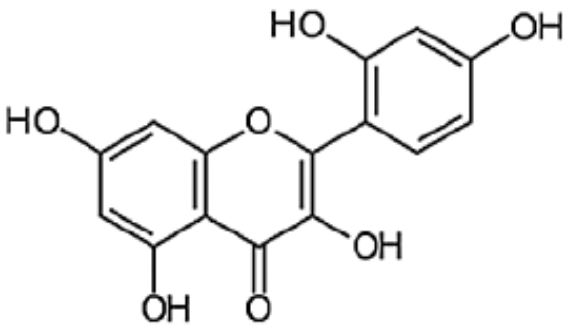



**Fig. 3:** GC-MS chromatogram of hexane extract of *C. pepo* leaves.


Phytol



Tritriacontane




Morin

Fig. 4: Chemical structure of bioactive compounds of hexane extract of *C. pepo* leaves.

Saponins are considered as defense system of plants and are reported to show many medicinal properties like antimicrobial, immunostimulant, hypocholesterolaemic, analgesic, antidiabetic, anti-inflammatory and anticarcinogenic properties [16]. The terpenoids have shown to decrease blood sugar level in animal models [17]. Steroids, terpenoids and saponins showed the analgesic properties and central nervous system activities [5,17,18]. In this study, ethylacetate extract of *C. pepo* leaves revealed the presence of phytochemicals such as steroids, saponins, proteins, cardiac glycosides and terpenoids. Hexane extract of *C. pepo* exhibited the presence of steroids, flavanoids, saponins, glycosides, cardiac glycosides and terpenoids. The presence of these secondary metabolites in both extracts of *C. pepo* leaf is quite instructive as this lends credence of the use of the plant for medicinal purpose.

The chromatogram of GC-MS of ethylacetate extract of *C. pepo* showed 7 peaks. Among the 7 compounds identified from the ethylacetate extract, α-amyrin is medically important. It is reported to possess anti-inflammatory [19-21], analgesic [22,23] and antifungal activities [24]. In the hexane extract of *C. pepo,* four peaks were identified; among these, 3 compounds *viz.,* phytol, α-amyrin and morin are medically important. Phytol is used as a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases [25]. Phytol is also a key acyclic diterpene alcohol that is a precursor for vitamins E and K1 and an antioxidant and a preventive agent against epoxide-induced breast cancer carcinogenesis [26]. Further it is reported to have many potential industrial uses and it could be used as a precursor in the synthesis of α-tocopherol (Vitamin E) [27] and phylloquinone (Vitamin K1) [28] as well as its use in production of cosmetics, fragrances and household cleaners [29]. Morin is a phytocompound that comes under the flavanoid group which showed protective effect against gastric ulcer by enhancing antioxidant enzyme activity leading to an accelerated ulcer healing [30]. Morin also possesses anticancer properties against human leukemic cells (U937 cells) [31], antioxidant and antibacterial activities [32].

The anti-inflammatory, antispasmodic, analgesic and diuretic effects can be attributed to their high alkaloids, phenols, tannins and flavonoids. The results evidently specifies that ethylacetate and hexane extract of *C. pepo* leaves contains yet various bioactive compounds that have various other medicinal properties that can be explored for the treatment of many diseases. However, isolation of individual phytochemical constituents and subjecting them to biological activity will definitely give fruitful results. Therefore, it may be recommended as a plant for phytopharmaceutical importance.

## ACKNOWLEDGEMENTS

The authors thank the Management, the Principal and Head of the Department of Zoology, Nehru Memorial College, Puthanampatti, Tiruchirappalli District, Tamilnadu, India for providing necessary facilities to do this research work successfully and also thank the University Grants Commission, New Delhi, for awarding Rajiv Gandhi National Fellowship (RGNF- SRF) as financial support.

## REFERENCES

[1] Sofowara, A.: Medicinal plants and Traditional Medicine in Africa. Wiley and Sons Ltd., New York (1982).
[2] Alade, P.I. and Irobi, O.N.: J. Ethanopharmacol., 39: 19-139 (1993).
[3] Brantner, A. and Grein, E.: J. Ethanopharmacol., 44: 35-40 (1994).
[4] Argal, A. and Pathak A.: J. Ethanopharmacol., 106: 142-145 (2006).
[5] Kubmarawa, D., Khan, M.E., Punah, A.M., and Hassan, M.A.: J. Med. Plants Res., 2(12): 352-355 (2008).
[6] Savithramma, N., Linga Rao, M. and Suhrulatha, D.: Middle East J. Sci. Res., 8: 579-584 (2011).
[7] Rupasinghe, H.P., Jackson, C.J., Poysa, V., Berado, C.D., Bewley, J.D. and Jenkinson, J.: J. Agric. Food Chem., 51(20): 5888-5894 (2003).
[8] Horborne, J.B.: Phytochemical Method, A guide to modern technique of plant analysis. 3rd ed. Chapman and Hall, New York (1998).
[9] Kokate, C.K.: A text book of practical pharmacognosy. 5th ed. Vallabh Prakashan, New Delhi (2005).
[10] Ansari, M. and Khodagholi, F.: Curr. Neuropharmacol., 11(4): 414-429 (2013).
[11] Sulaiman, S., Ibrahim, D., Kassim, J. and Sheh-Hong, L.: J. Chem. Pharm. Res., 3(4): 436-444 (2011).

[12] Abaoba, O.O. and Efuwape, B.: Biol. Res. Comm.. 13: 183-188 (2001).
[13] Tomczyk, M., Pleszczyńska, M. and Wiater, A.: Molecules, 15(7): 4639-4651 (2010).
[14] Tapas, A.R., Sakarkar, D.M. and Kakde, R.B.: Trop. J. Pharma. Res., 7: 1089-1099 (2008).
[15] Dolara, P., Luceri, C., De Filippo, C., Femia, A.P., Giovannelli, L., Carderni, G., Cecchini, C., Silvi, S., Orpianesi, C. and Cresci, A.: Mutation Res., 591: 237-246 (2005).
[16] Morrissey, J.P. and Osbourn, A.E.: Microbiol. Mol. Biol. Rev., 63: 708-724 (1999).
[17] Mandal, S.C., Maity, T.K., Das, J., Saba, B.P. and Pal, M.: J. Ethanopharmacol., 72: 87-92 (2009).
[18] Shaik, T., Rub, R., Kiran, B., Pimprikar, R.B. and Sufiyan, A.: J. Pharm. Sci. Res., 2(1): 41-44 (2010).
[19] Recio, M.C., Giner, R.M., Manez, S. and Rios, J.L.: Planta Med., 61(2): 181-185 (1995).
[20] Madeiros, R., Otuki, M.F., Avellar, M.C. and Calixto, J.B.: Eur. J. Pharmacol., 55(9): 227-235 (2007).
[21] Okoye, N.N., Ajaghaku, D.L., Okeke, H.N., Ilodigwe, E.E., Nworu, C.S. and Okoye, F.B.S.: Pharm. Biol. 52(11): 1478-1486 (2014).
[22] Otuki, C., Ferrareira, J., Lima, F.V., Meyre-Silva, C., Malheiros, A., Muller, L., Cani, G.S., Santos, A.R., Yunes, R.A. and Calixto, J.B.: J. Pharmacol. Exp. Therap., 31(1): 310-318 (2005).
[23] Soldi, C., Pizzolatti, G., Luiz. A., Marcon, R., Meotti, F., Miotob, L. and Santos, A.: Bioorg. Med. Chem. 16(6): 3377-3386 (2008).
[24] Batovska, A.A., Todorova, I.T., Nedelcheva, D.V., Parushev, S.P., Atanassov, A.J., Hvarleva, T.D., Djakova, G.J. and Bankova, V.S.: J. Plant Physiol., 165: 791-795 (2008).
[25] Ogunlesi, M., Okiei, W., Ofor, E. and Osibote, A.E.: African J. Biotech., 8: 7042-7050 (2009).
[26] Yu, F., Gapor, A. and Bender, W.: Cancer Detect. Prev., 29: 383-388 (2005).
[27] Netscher, T.: Vitam. Horm., 76: 155-202 (2007).
[28] Daines, A.M., Payne, R.J., Humphries. M.E. and Abell, A.D.: Curr. Org. Chem. Toxicol., 7: 1625-1634 (2003).
[29] McGinty. D., Letizia, C.S. and Api, A.M.: Food Chem. Toxicol., 48: S59-S63 (2010).
[30] Sarkar, S., Sengupta, A., Mukhrjee, A., Guru, A., Patil, A., Kandhare, A.D. and Bodhankar, S.L.: Pharmacologia, 6(7): 273-281 (2015).
[31] Park, C., Lee, W.S., Se-II. Go., Nagappan, A., Han, M.H., Hong, S., Kim, G.S., Kim, G.Y., Kwon, T.K., Ryu, C.H., Shin, S.C. and Choi, Y.H.: Int. J. Mol. Sci., 16(1): 645-659 (2015).
[32] Yang, J. and Lee, H.: J. Korean Soc. Appl. Biol. Chem., 55: 485-489 (2012).