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Research Article

ISOLATION AND IDENTIFICATION OF FLAVONOIDS FROM *TINOSPORA CORDIFOLIA*

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ABSTRACT

Tinospora cordifolia is an important medicinal plant belonging to family Menispermaceae. Flavonoids were isolated from leaves, stem, seeds and *in vitro* callus of the species. The dried samples were separately soxhlet extracted in 80% methanol and then re-extracted with petroleum ether, diethyl ether and ethyl acetate. The fraction was concentrated and subjected to thin layer chromatography. The Rf value of isolated compounds and standard was calculated, which confirm the presence of quercetin, luteolin and kaempferol. The purified material was subjected to its IR spectra, High-performance liquid chromatography peak of isolated quercetin at retention time 2.652 min was observed. This study is also of practical importance because flavonoids are an important ingredient of *T. cordifolia.*

INTRODUCTION

Flavonoids (from the Latin word *flavus* meaning yellow) are a class of plant secondary metabolite. Flavonoids are widely dispersed in plants, full filling many functions. Flavonoids are responsible for plant pigmentation producing yellow or red/blue pigmentation in petals to attract pollinator animal. In higher plants, flavonoids are also significant in UV filtration, symbiotic nitrogen fixation and floral pigmentation. Flavonoids also have the properties such as chemical messengers, physiological regulators, and cell cycle inhibitors. Flavonoids have been reported as serving multiple functions in plants (Shirley *et al* 1996).They are capable to absorb solar wavelengths (i.e., UV-B and UV-A), slow down the generation of ROS, and reduce ROS once they are formed (Agati *et al* 2012).

Flavonoids carry out functional roles of remarkable significance in plant-environment interactions. Flavonoids may regulate auxin movement, the ability of flavonoids to create auxin gradients translates into phenotypes with different morphoanatomical features (Taylor *et al* 2005). Auxin movement control by flavonoids may have huge value in the stress-induced morphogenic responses of plants such as the flight strategy of sessile organisms exposed to unfavorable environments (Jansen *et al* 2002).

Foods with flavonoid content include black tea, green tea and blue berries (Ayoub *et al* 2016). Peanut skin contains significant polyphenol content, including flavonoids (de Camargo *et al* 2015). Flavonoids consist polyphenolic compounds having a benzo-γ-pyrone structure and their synthesis occur by phenyl propanoid pathway. Available reports tend to show that flavonoids are responsible for the variety of pharmacological activities (Mahomoodally *et al* 2005, Pandey *et al* 2007). Flavonoids have been shown to have a broad range of biological and pharmacological activities in *in vitro* studies include anti-allergic (Yamamoto *et al* 2001) anti-inflammatory and antioxidant (Cazarolli *et al* 2008), antimicrobial (Gurgueira *et al* 2011, Manner *et al* 2013) antifungal and antiviral (Friedman 2007, Aires *et al* 2017), anticancer (de Sousa *et al* 2007) and anti-diarrheal (Schuier *et al* 2005) activity. Inflammation has been implicated as a possible origin of numerous local and systemic diseases, such as cancer (Ravishankar *et al* 2013) cardiovascular disorders (Manach *et al* 2005) diabetes mellitus (Babu *et al* 2013) and celiac illness (Ferretti *et al* 2012). Preliminary studies show that flavonoids may have an effect on anti-inflammatory mechanisms via their capability to inhibit reactive oxygen and nitrogen compound (Izzi *et al* 2012). More than 2000 flavonoids have been reported plants (Harborne, 1980). TLC, UV and IR spectral studies have provided new scope to the chemistry of flavonoids to such an extent that their occurrence have become essential taxonomically (Smith, 1969).Presence

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of flavonoids has been reported from many plant species like *Lyciumbarbarum* (Harsh *et al* 1983), *Passifloraplamer* (Ulubelen *et al* 1984),*Cassia angustifolia* (Goswami and Reddi 2004) *Jatropacurcas* L.(Saxena *et al* 1984) *Launaeprocumbens* (Reddy *et al* 2012), *Hiptagebenghalesis* (Yadav *et al* 2012) and *Sesbaniaesban* (Mythili *et al* 2013).

Tinospora cordifolia is a large, glabrous, deciduous, climbing shrub. it has different types of secondary metabolites likes alkaloids, diterpenoid, lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides (Meshram *et al* 2013). Flavonoids, glycosides, saponins and some amount of phytosterols also found in it. These active constituents are responsible for the observed antioxidant activity (Onkar *et al* 2012).

The present study deals with the isolation and identification of flavonoids from *in vivo* (leaves, stems, and seeds) and *in vitro* callus tissue of *T.cordifolia.*

MATERIALS AND METHODS

In vivo

Different plant parts of *T.cordifolia* (stem, leaves and seeds) collected locally were dried, powderedand used for extraction along with the *in vitro* tissue samples as described (Chaturvedi and Sharma, 2017).

In vitro

Six week old callus tissue (callus induced from nodal stem segments) of *T.cordifolia* grown on MS medium(Murashige and Skoog, 1962) supplemented with 6-benzylaminopurine and α-naphthalene acetic acid (1:0.2 mg/l) was dried in an oven at 100°C for 15 min. to inactivate enzymes followed by 60°C till a constant weight was achieved. Tissue samples were powdered and used for extraction.

Extraction

Different plant parts (seeds, stem and leaves) along with callus tissue of *T.cordifolia* were air dried and powdered, separately. Each of these extracted separately with 80% methanol on water bath (Subramanian andNagarajan, 1969) for 24 h. The methanol soluble fractions were filtered, concentrated *in vacuo* and aqueous fractions were fractioned by sequential extraction with petroleum ether (Fr-1), diethyl ether (Fr-II) and ethyl acetate (Fr-III) separately. Each step was repeated thrice for complete extraction, fraction 1 was discarded in each case because it contained fatty substance, whereas fraction-II and fraction-III were concentrated and used for determining flavonoids.

Fraction-III was further hydrolyzed by refluxing with 7% sulphuric acid ($10mLg^{-1}$ plant material for 2 h), filtered and filtrate was extracted thrice with ethyl acetate. All ethyl acetate layers were pooled separately, neutralized by distilled water and concentrated *in vacuo.* Before chromatographic examination.both fraction-II and-III were taken up in small volume of ethanol (2-5mL).

Thin Layer Chromatography (TLC)

Thin glass plates (20x20 cm) were layered with Silica gel G (250mμ thick). The freshly prepared plates were air dried at room temperature; thereafter these were kept at 100^0C for 30 min to activate and then cooled at room temperature. The freshly prepared and activated plates were used for analysis. Each of the extract was co-chromatographed with authentic flavonoid as a marker. (quercetin, luteolin, kaempferol).These plates were developed in an air tight chromatographic chamber saturated with solvent mixture (Benzene: Acetic Acid: Water:: 125:72:3; Wong and Francis, 1968). The developed plates were air dried up and visualized under UV light by exposure to ammonia fumes. The mouth of a 100 mL containing concentrated NH_4OH was held in contact with each spot for about 5-10 seconds and fluorescent spots corresponding to that of standard markers were marked. The developed plates were also sprayed with 5% $FeCl_3$, 0.1% alcoholic $AlCl_3$ and kept in I_2 chamber separately. The colored spots were noted and the Rf value of each spot was calculated. Solvent systems such as n-butanol, acetic acid, water (4:1:5), tertiary butanol, acetic acid, water (3:1:1) were also tested, but the solvent system containing benzene, acetic acid, water (125:72:3) gave better results.

Preparative thin layer chromatography (PTLC)

PTLC of aforementioned flavonoid extracts was carried out using silica gel G coated plates (BDH; 500mμ in thickness) by spotting the extract as well as standard markers (luteolin, kaempferol and quercetin). These plates were developed in the solvent mixture of benzene, acetic acid, and water (125:72:3), air dried and examined under UV light. Each of spots corresponding with the standard markers were marked, scraped from 200 plates, and eluted with 50% methanol. The eluted fractions were filtered, air dried and again co-chromatographed along with standard markers to test their purity. The eluted fractions were subjected to crystallization separately and melting point (mp), mixed melting point (mmp) was determined. The isolates were also subjected to ultraviolet and infrared spectral studies. This purified material was also subjected to HPLC studies.

HPLC

For high performance liquid chromatography **(**HPLC) analysis binary pump assembly (Elico Private Limited with C-18 Column), the following conditions were applied: 1 ml/min flow rate, 20 μl of sample injected, detection at λ 202 nm, and HPLC crude methanol (100) as the eluent. Before injection, the extract was dissolved in 1 ml absolute methanol. Method validity was assessed based on all test parameters to cover the range of samples and concentration involved. Tests were done in triplicates. A standard curve was generated from integrated peak area and concentration of the same standard expressed as percent recovery of the sample.

Identification

Melting point, mixed melting point, Rf value and IR spectra of each of the isolated compound was taken and also on the basis of preliminary detection and confirmation with standard compounds.

RESULTS

The developed plates were sprayed with 5% ethanolic ferric chloride solution it showed spots which coincided with that of the reference quercetin, when plates were placed in a chamber saturated with ammonia vapors, it also showed deep yellow

color of quercetin. Rf value (0.78) of quercetin isolated from the samples coincided with the Rf value of standard quercetin. Quercetin was detected in all the *in vivo* (seeds, leaves, stem; Figs.1-3) and *in vitro* callus (Fig.4) samples of *T.cordifolia.* Kaempferol, Luteolin are also identified by melting point, mixed melting point,Rf value(Table 1) and IR spectra of each of the isolated compound (Figs.5-7)When isolated quercetin was subjected to HPLC (Fig. 8), it showed retention time2.652 min which coincided with that of standard quercetin (Fig.9).

Fig 1 TLC plate of flavonoids of seeds extract

Fig 2 TLC plate of flavonoids of leaves extract

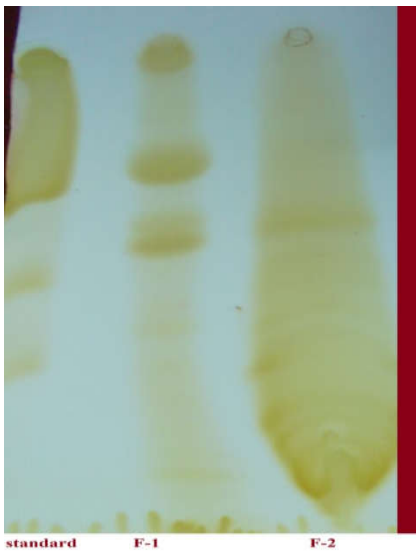

Fig 3 TLC plate of flavonoids of stem extract

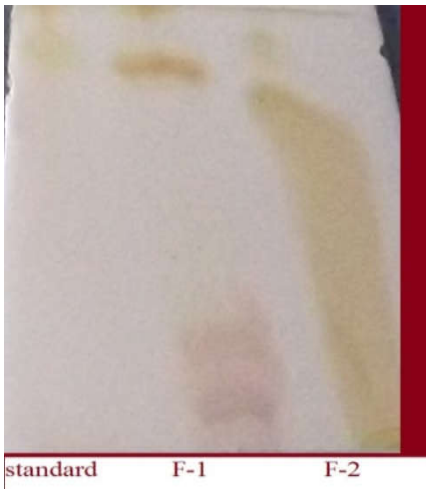

Fig 4 TLC plate of flavonoids of callus extract

Table 1 Chromatographic behavior and physiochemical characteristics of isolated flavonoids

Isolated Compounds	R_f value			Physical appearance			Color after spray				Melting point	IR Spectral Peak V (KBr) cm^{-1}
	S_1	S_2	S_3	Day Light	UV ammonia	Iodine Vapor	R_1 Visible	R_1 UV	R_2 Visible	R_2 UV		
Kaempferol	0.86	0.83	0.55	GN-YW	BT-YW	YW-BN	BN	BK	YW	YW-GN	276-278	(O-H) (3410 cm^{-1}(270,295,344, 1690
Luteolin	0.56	0.83	0.77	GN-YW	YW	YW-BN	TN	BK	DL-YW	YW-GN	326-329	3421,2965, 1736(lactone), 1510(furan) 1461,1388, 1360,1274, 1242,1187, 1136,1028 903,850cm^{-1}
Quercetin	0.78	0.64	0.41	GN-YW	YW	YW-BN	BT-GY	BK	DL-YW	YW-GN	315-320	3423,1739,1655 (0-H), 1508,1305,1203 (C=C),1088

Abbreviations:- S_1-benzen: acetic acid: water (125:73:3),S_2-n-butanol:acetic acid: water (4:1:5),S_3-Conc.HCl:acetic acid :water (3:30:10), R_3-5% $feCl_3$ solution, R_2-5% alc.$AlCl_3$ solution, YW-yellow, BK-black, BN-brown, BT-bright, DL-dull GN-green,GY-gray.

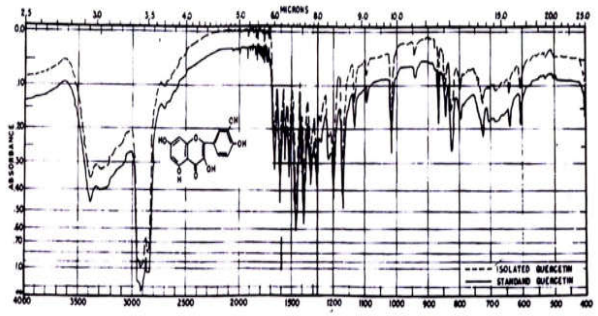

Fig 5 Infrared spectra of standard and isolated Quercetin

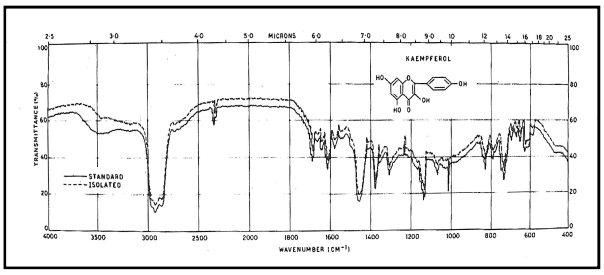

Fig 6 Infrared spectra of standard and isolated Kaempferol

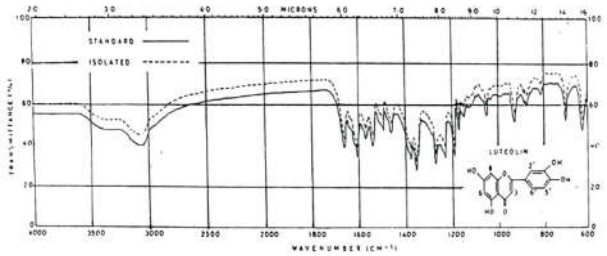

Fig 7 Infrared spectra of standard and isolated Luteolin

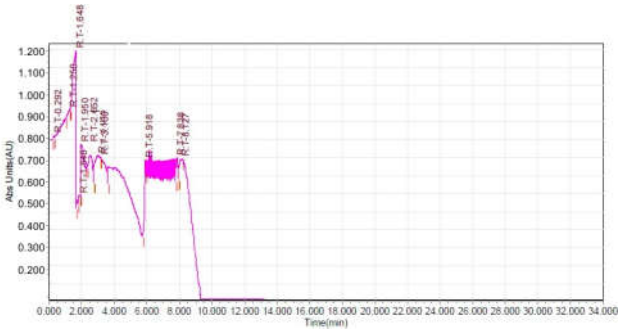

Fig 8 HPLC chromatogram of seed extract

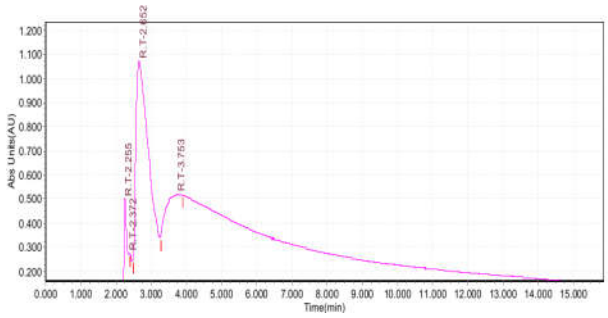

Fig 9 HPLC chromatogram of standard Quercetin

DISCUSSION

This study confirms the presence of flavonoids in the leaves, seeds, stem and *in vitro* grown callus tissue of *T.cordifolia*.

Quercetin is a plant polyphenol from the flavonoid found in many fruits, vegetables, leaves, and grains. It can be used as an ingredient in supplements, beverages, or foods. Quercetin has been reported to inhibit the oxidation of other molecules and hence is classified as an antioxidant (Russo *et al* 2014).Kaempferol is a natural flavonol found in a variety of plants and plant-derived foods. Luteolin, a flavonoid with Potential for Cancer Prevention and Therapy. Several studies have shown kaempferol have a wide range of pharmacological activities, including antioxidant (Calderon *et al* 2011), anticancer (*Nöthling et al* 2007), antidiabetic (Veeresham *et al* 2014) cardio-protective (Khalil *et al* 2010).

As mentioned earlier, flavonoids (Quercetin, Kaempferol, Luteolin) have anti-inflammatory ,antioxidant and anticancer properties. So the antioxidant, anti-inflammatory and anti-carcinogenic activities of *T.cordifolia* may be attributed to the presence of the flavonoids obtained from the plant. The propose mechanism of action range from scavenging reactive oxygen species to inhibiting enzymes responsible for cancer promotion and progression. They have the ability to block the release of the enzymes responsible for pain and inflammation; this supports the use of *T.cordifolia* in herbal medicine for the treatment of arthritis, elephtiasis and muscular dystrophy.

In the present life styles, where stress conditions are common, leading to excess production of free radicals, these natural products will prove a support to our biological system to sustain and balance metabolism. These findings supported the use of *T.cordifolia* in phyto medicine as antioxidant and anti-inflammatory agent for disease treatment and prevention. Isolation and extraction of these compounds *in vivo* (leaves, stem and seeds) and *in vitro* callus from *T.cordifolia* determines the value of plant, which could be of significant interest to the development of new drugs.

CONCLUSION

From the present work, it has been concluded that flavonoids have been extracted successfully from the *in vivo*and *in vitro* plant parts of *Tinospora cordifolia*. The identification of flavonoids was attempted by direct comparison with their Rf values. The isolated compounds were identified through FT-IR spectroscopy.

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References

Aires A, Dias C, Carvalho R, and Saavedra M, (2017). Analysis of glycosylated flavonoids extracted from sweet-cherry stems, as antibacterial agents against pathogenic Escherichia coli isolates. *Acta BiochimicaPolonica,* 64(2):265-271.

Ayoub M, De Camargo AC and Shahidi F, (2016). Antioxidants and bioactivities of free, esterified and insoluble-bound phenolics from berry seed meals. *Food Chemistry*, 197(Pt A): 221-232.

Babu PV, Liu D and Gilbert ER, (2013).Recent advances in understanding the anti-diabetic actions of dietary

flavonoids. *The J. Nutritional Biochemistry*, 24(11): 1777-1789

Calderon-Montaño JM, Burgos-Moron E, Perez-Guerrero C and Lopez-Lazaro M, (2011).A review on the dietary flavonoid kaempferol. *Mini Rev. Med. Chem*, 11(4): 298-344.

Casteel HW, and Wender SM, (1953). Identification of flavonoid compounds, Rf values and color tests. *Anal.Chem*, 25: 508.

Cazarolli LH, Zanatta L, Alberton EH, Figueiredo MS, Folador P, Damazio RG, Pizzolatti MG, and Silva F.R, (2008). Flavonoids: Prospective Drug Candidates. Mini-Reviews in Medicinal Chemistry, 8(13): 1429-1439.

Chaturvedi P and Sharma VK, (2017).Phytosterols from *Tinospora cordifolia in vivo* and *in vitro* study. *Int. J.Pharma Bio. Sci.,* 8(3B): 557-563

De Camargo AC, Regitano-d'Arce MA, Gallo CR and Shahidi F, (2015).Gamma-irradiation induced changes in microbiological status, phenolic profile and antioxidant activity of peanut skin. *J. Functional Foods*, 12: 129-143.

De Sousa RR, Queiroz KC, and Souza AC, (2007). Phosphoprotein levels, MAPK activities and NFkappaB expression are affected by fisetin. *J. Enzyme Inhib.Med.Chem,* 22(4):439-444.

Ferretti G, Bacchetti T, Masciangelo S and Saturni L, (2012).Celiac Disease, Inflammation and Oxidative Damage. A Nutrigenetic Approach. *Nutrients*, 4(12):243-257.

Friedman M, (2007). Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. *Molecular Nutrition and Food Research*, 51(1): 116-134.

G. Agati E, Azzarello S, Pollastri M and Tattini, (2012). Flavonoids as antioxidants in plants: location and functional significance. *Plant Science*, 196: 67-76.

Gaurav J, Mishra and Reddy MN, (2012). Preliminary phytochemical screening and antibacterial analysis of the leaf extracts of *launaea procumbens Roxb. Int. J. Phytopharmacology*, 3(2): 147-151.

. Goswami A and Reddi A, (2004).Antimicrobial activity of flavonoids of medicinally important plant *Cassia angustifolia in vivo* and *in vitro*. *J. Phyto. Res*, 17(2): 179-181.

Cushnie TP and Lamb AJ, (2011). Recent advances in understanding the antibacterial properties of flavonoids. *Int. J. Antimicrobial Agents*, 38(2): 99-107.

Harborne JB, (1986). Nature, distribution and function of plant flavonoids, in plant flavonoids in biology and medicine. In:Biochemical, Pharmacological and Structure-Activity Relationships (Eds.) Cody, V, Middleton, E, Jr and Harborne, JB, Alan R Liss, Inc. New York, pp. 15-24.

Harsh ML, Nag TN and Jain S, (1983). Arid zone plants of Rajasthan a source of antimicrobials. Com. Phys. Eco, 8(2): 129-131.

Izzi V, Masuelli L, Tresoldi I, Sacchetti P, Modesti A, Galvano F and Bei R, (2012). The effects of dietary flavonoids on the regulation of redox inflammatory networks. Frontiers in Bioscience (Landmark edition), 17(7): 2396-2418.

Jansen MAK, (2002). Ultraviolet-B radiation effects on plants: induction of morphogenic responses. *Physiologia Plantarum,* 116: 423-429.

Khalil MI and Sulaiman SA, (2010).The Potential Role of Honey and its Polyphenols in Preventing Heart Diseases: A Review, Afr. J. Tradit. Complement Altern. Med, 7(4): 315-321.

Taylor LP and Grote old E, (2005). Flavonoids as developmental regulators, Current Opinion in Plant Biology, 8(3): 317-323.

Mahomoodally MF, Gurib-Fakim A and Subratty AH, (2005).Antimicrobial activities and phytochemical profiles of endemic medicinal plants of Mauritius. *Pharmaceutical Biology*, 43(3): 237-242

Manach C, Mazur A and Scalbert A, (2005). Polyphenols and prevention of cardiovascular diseases. *Current Opinion in Lipidology,* 16(1): 77-84.

Manner S, Skogman M, Goeres D, Vuorela P and Fallarero A, (2013). Systematic exploration of natural and synthetic flavonoids for the inhibition of *Staphylococcus aureus* Biofilms. *Int. J. Molecular Sciences*, 14(10): 19434-19451.

Meshram A, Bhagyawant SS, Gautam S and Shrivastava N, (2013).Potential Role of *Tinospora cordifolia* in Pharmaceuticals. World J. Pharm.Sci., 2(6): 4615-4625.

Murashige T and Skoog F, (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures, *Physiol. Plant*, 15: 473-497.

Mythili T and Ravindhran R, (2013). Determination of Quercetin by HPTLC Method in *Sesbaniasesban* (L.) Merr. Stem Extract, IJAPBC, 2(1): 113-119.

Nöthlings Ute, Murphy, Suzanne P, Wilkens, Lynne R, Henderson, Brian E, Kolone and Laurence N, (2007).Flavonols and Pancreatic Cancer Risk. American J. Epidemiology, 166(8):*924-931*.

Onkar P, Bangar J and Karodi R, (2012). Evaluation of Antioxidant activity of traditional formulation Giloysatvas and hydroalcoholic extract of the Curculigoorchioidesgaertn*. J. App. Pharma. Sci*, 2(6): 209-213.

Pandey AK, (2007). Anti-staphylococcal activity of a pan-tropical aggressive and obnoxious weed Pariheniumhisterophorus: an in vitro study. National Academy Science Letters, 30: 383-386.

Ravishankar D, Rajora AK, Greco F and Osborn HM, (2013). Flavonoids as prospective compounds for anti-cancer therapy", *The Int. J. Biochemistry & Cell Biology,*45: 2821-2831.

Russo GL, Russo M, Spagnuolo C, Tedesco I, Bilotto S, Iannitti R and Palumbo R, (2014).Quercetin: A Pleiotropic Kinase Inhibitor Against Cancer. *Cancer Treatment and Research,* 159: 185-205.

Saxena S, Sharma R, Rajore S and Batra A, (2005).Isolation and identification of flavonoids "Vitexin" from *Jatropha curcas L. Jour. Pl.Sci.Res*., 21:116-117.

Schuier M, Sies H, Illek B and Fischer H, (2005). Cocoa-Related Flavonoids Inhibit CFTR-Mediated Chloride Transport across T84 Human Colon Epithelia*, J. Nutr,* 135(10): 2320-2325.

Shirley BW, (1996). Flavonoid biosynthesis:"new" functions for an "old" pathway, Trends in Plant Science, 1: 377-382.

Smith EB, (1969). In: Prospective in Phytochemistry (Eds) Harborne JB and Swain T, Academic Press, London.

Subramanian SS and Nagarajan, (1969).Flavonoids of seeds of *Crotalaria retusa* and *C. straita. Current Science*, 38:365.

Ulubelen A, Mabry JJ, Dellamonicas G and Chopin J, (1984).Flavonoids of *Passiflora palmer. J. Nat. Prod*, 47(2):384-385.

Veeresham C, Rama Rao A and Asres, (2014). Aldose Reductase Inhibitors of Plant Origin. *Phytother Res,* 28(3): 317-333.

Wong E and Francis CM, (1968). Flavonoids in genotypes of *Trifolium subterranean*. The normal flavonoids pattern of the geralation variety. *Phytochemistry*, 7(12):2123-2129.

Yadav S and Kumar P, (2012).Production, isolation and identification of flavonoids from aerial parts of *Hiptagebenghalensis. Int. J. Life Science and Pharma Research,* 2,L-1-L-2.

Yamamoto Y and Gaynor RB, (2001). Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. *J. Clinical Investigation*, 107(2): 135-142.

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