Research Article

Phytochemical Analysis and Anti-cancer Activity of *Rhinacanthus nasutus*

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ABSTRACT

Cancer is a condition in which the body cells multiply uncontrollably. It is a deadly disease that develops over the time and it is defined by persistent, aberrant and relatively autonomous cell proliferation as a result of a permanent cellular defect that is handed down to the progeny. It is one of the most common diseases in human population and ongoing identification of new anticancer medicines from natural sources is of great scientific and commercial importance. New, effective and inexpensive anti-cancer treatments are constantly in demand. Traditional medicine is an important and alternative resource for discovering new anti-cancer drugs. One such a medicinal plant *Rhinacanthus nasutus* (L) (Acanthaceae) comprises three main alkaloids: *Rhinacanthin C, Rhinacanthin D* and *Rhinacanthin N*. *Rhinacanthin* has been considered to have the ability to stop the aberrant cell growth and development. However, no scientific reports on cancer disease has been published. Hence in the present study focused on analysis of phytochemicals in aqueous extract of *Rhinacanthus nasutus*. The phytochemical analysis of *R.nasutus,* revealed the presence of alkaloids, phenols, saponins, flavonoids, tannins, steroids and terpenoids except cardiac glycosides. The aqueous extract of *Rhinacanthus nasutus* possesses a higher concentration of phenolic compounds and flavonoids, according to quantitative phytochemical study. It also possesses the good free radical scavenging activity. Hence the present study, focussed on analysis of phytochemicals and free radical scavenging activity of *R.nasutus,* will be useful in the synthesis and preparation of new drugs of pharmaceutical importance.

Key words: Antioxidant activity, Leaves extract, Medicinal plants, Phytochemicals, *Rhinacanthus nasutus.*

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INTRODUCTION

Cancer is one of the most common non-communicable diseases in developing countries and it is the world's second biggest causes of death after cardiac diseases, with the burden growing by the day.[1] Cancer has been observed to be more common in India in recent years and early detection is critical to overcome the condition successfully. Herbal medicines, which are produced from plants, have played an important role in health care system both in ancient time and in modern societies. Herbal medications are now widely used in underdeveloped nations to reduce cancer symptoms and treatment with few adverse effects.

Plants have served as models for 25 to 50 percent of Western medications. Many commercially validated medications utilized in modern medicine were first used in crude form in traditional or folk medicinal practices, or for other uses that suggested biological activity. Plant-based medications are often safer than synthetic equivalents and they provide significant therapeutic benefits at a lower cost.[2] Many plant extracts have been found to be effective antimicrobial agents and active components found in these extracts may provide a new foundation for anti-cancer and antimicrobial chemicals.[3] Crude plant extracts in the form of decoction, tincture or herbal extract have long been used in herbal therapy

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to treat the variety of ailments. Phytochemicals found in plant-derived products have been shown to have antibacterial, antioxidant, anti-mutagenic, anti-carcinogenic, antithrombotic, and vasodilatory properties.[4] Plant-derived antioxidants including stilbenes, coumarins, tannins, lignans, quinones, flavones, flavonols, xanthones, phenolic acids, catechins, proanthocyanins and anthocyanins have redox characteristics that allow them to act as hydrogen donors, reducing agents, antioxidant, hydroxyl radicals (OH.) or superoxide radical (O_2.) scavengers.[5] In addition to antioxidant action, various studies have shown that phenols and/or phenolic extracts have antibacterial activity, making them a good alternative or supplement to conventional antibiotics. The ancient attribution of nutrition's role in cancer has been widely acknowledged in scientific literature regarding carcinogenesis and the interrelationship between nutrition and cancer incidence and recurrence.[6]

In cancer cells, several medicinal herbs are found to trigger apoptosis through a variety of mechanisms. When compare to other medication sources, traditional medicine has contributed several unique therapeutic substances for preventive and curative medicine. Vinca alkaloids (vinblastine and vincristine), taxanes (paclitaxel and docetaxel), podophyllotoxin and its derivatives are all medicinal plant components (topotecan and irinotecan). Plant-derived anticancer drugs called camptothecins have been tested in clinical trials.[7] Several studies have found that secondary metabolites such as alkaloids, polyphenols and terpenes have anti-mutagenic and anticancer activities.[8] As a result, alternative therapeutic approaches to combat the terrible disease known as cancer are critical. WHO (World Health Organization) has taken significant steps to do research on medicinal plants that are more active and have fewer adverse effects. Hence the present study focused on exploration of phytochemicals in *Rhinacanthus nasutus* which will be useful in synthesis and preparation of new anticancer drugs in pharmaceutical industries.

MATERIALS AND METHODS

Collection and preparation of plant sample

Rhinacanthus nasutus was collected from the waste lands in and around Siruseri, Kanchipuram district, Chennai, Tamil Nadu, India, where it was found naturally (Figure 1). The fresh leaves of *Rhinacanthus nasutus* was collected and stored. The fresh leaves are soaked with water for 24 hr and then filtered. The filtered supernatant was collected in reagent bottle, closed tightly and stored at 4°C until the time of use.

Figure 1: Habit of *Rhinacanthus nasutus*. (A) Habit (B) Flowering stage (C) Fruiting stage (D) Roots.

Determination of suitable solvents for extraction of *Rhinacanthus nasutus*

The dried powder sample of *Rhinacanthus nasutus* was subjected to different solvents to find their solubility. Solvent like water, alcohol, acetone, Dimethyl sulfoxide (DMSO), ethyl acetate, chloroform and hexane were used and their solubility was observed. The pH values were also observed.

Phytochemical Screening

The preliminary qualitative phytochemical screening in aqueous extract of *Rhinacanthus nasutus* was done to find out the different phytochemical constituents such as alkaloids, phenolic compounds, flavonoids, saponins, tannins, glycosides, steroids, carbohydrates, proteins and terpenoids using standard method.[9,10]

Test for alkaloids

About 50 mg solvent-free aqueous extract was stirred with 5 ml of dilute hydrochloric acid and filtered. To the filtrate, 2 ml of Hager's reagent (aqueous solution of picric acid) was added. A yellow precipitate appears, that indicated the presence of alkaloids.[11]

Test for phenolic compound

About 50 mg of the aqueous extract was dissolved in 5 ml of distilled water. To this, a few drops of 5% neutral ferric chloride solution was added. Phenolic compounds were indicated by the presence of dark green colour.[12]

Test for tannins

The aqueous extract (500 mg) was added into 10 ml of freshly prepared 10% potassium hydroxide (KOH) in a beaker and shaken well to dissolve. A dirty precipitate formation indicated the presence of tannins in the sample.[13]

Test for flavonoids

An aqueous solution of the plant extract was treated with 10 % ammonium hydroxide solution. The appearance of bulky white precipitate indicated the presence of flavonoids.[14]

Test for terpenoids

About 50 mg of the aqueous extract was added to 1mL of chloroform. It was mixed well and added to acetic anhydride followed by concentrated sulphuric acid from the sides of the tubes. The appearance of red and bluish-green colour indicated the presence of steroids and triterpenoids.

Test for saponins

The aqueous extract (50 mg) was diluted with distilled water and made up to 10 ml. The suspension was shaken in a graduated cylinder for 15min; an increase in layer of foam indicated the presence of saponins.[15]

Test for glycosides

About 50mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hr on a water bath and filtered. To 2 ml of filtrate, 3 ml of chloroform was added and shaken well. The chloroform layer was separated and 10% ammonia solution was added to it. The formation of pink colour indicated the presence of glycosides.

Test for steroids determination

Two ml of chloroform and 1ml concentrated sulphuric acid were added to 10 drops of the aqueous extract was mixed with isopropyl alcohol, slowly until double phase formation. The presence of a dish-brown colour in the middle layer marks the presence of a steroidal ring

Estimation of total phenolics

The quantitative estimation of phenolics in the extract of *Rhinacanthus nasutus* was determined based on the Siddhuraju and Becker, (2003) method.[16] About 0.5 ml of 1N Folin- Ciocalteu reagent and 2.5 ml of 20% sodium carbonate solutions were added and then the volume was made up to 10 ml with water. Followed by 40 min dark incubation and the absorbance were recorded at 725nm against blank for the estimation of phenolics. The results were based on the calibration curve: $y = 0.029x - 0.065$, $R^2 = 0.955$ where x was the absorbance and y was the Gallic acid equivalents (mg/g) and were expressed in terms of milligrams Gallic acid equivalents (GAE) per gram of extract.

Determination of total Flavonoids

The total flavonoid content in the extract was estimated by the general procedure.[17] To each 300 µl of *Rhinacanthus nasutus* extracts 2 ml of distilled water was added followed by150 µl of $NaNO_2$. The contents of the tubes were subjected to incubation for 6 min at room temperature. After incubation 150 µl of $AlCl_3$ (10%) was added and incubated again for 6 min at room temperature. Then 2 ml of 4% NaOH was added, vortexed well and kept at room temperature for another 15 min. The absorbance of pink colour was read spectrophotometrically at 510 nm. The results were based on the calibration curve: $y = 0.002x + 0.006$, $R2 = 0.992$ where x was the absorbance and y was the rutin equivalents (mg/g) and the results were expressed in terms of milligrams rutin equivalents per gram of extract.

In vitro antioxidant assay

DPPH radical scavenging activity

The radical scavenging activity of the extract of *Rhinacanthus nasutus* was determined by the method of DPPH radical scavenging activity.[18] The sample extract at various concentrations was added to 5 ml of 0.1 mm methanolic solution of DPPH and allowed to stand for 20 min at 27°C. The absorbance of the solution was read at 517 nm using a spectrophotometer. Methanol was served as blank and a solution without powder extract of *Rhinacanthus nasutus* served as the negative control. The mixture of methanol, DPPH and standard rutin served as the positive control. The radical scavenging ability of the extract is expressed by IC_{50} value of the extract.

RESULTS

Physiochemical analysis of *Rhinacanthus nasutus*

Physiochemical characterization has become an important part of quality control in drug development both single and compound formulations. Because a medicine's effectiveness is largely determined by its physical and chemical properties. Determination of physiochemical features is critical for drug accuracy. It also aids in the identification of ingredients, which typically leads to the discovery of drug mechanism of action. Physiochemical evaluations such as solubility and pH values were determined in this study and the results were presented in Table 1.

Phytochemical Analysis of *Rhinacanthus nasutus*

Rhinacanthus nasutus is being used as the traditional medicine not only in different parts of India but also throughout the world for the time immemorial. The result of the phytochemical screening of aqueous extract of this plant directly correlates with the facts of using this plant as an ethno medicine. We detected the presence

Table 1: Physiochemical properties of aqueous extract of Rhinacanthus nasutus.

S. No	Characteristics	Observation
1	Color	Greenish white
2	Solubility in water	Easily soluble in water
3	Solubility in alcohol	Easily soluble in alcohol
4	Solubility in acetone	Easily soluble in acetone
5	Solubility in DMSO	Easily soluble in DMSO
6	Solubility in ethyl acetate	Insoluble
7	Solubility in chloroform	Insoluble soluble in chloroform
8	Solubility in hexane	Insoluble soluble in hexane
9	pH	6.7

Table 2: Preliminary phytochemicals screening of *Rhinacanthus nasutus.*

S.No	Phytochemical constituents	Presence or absence
1	Alkaloids	++
2	Phenolic compounds	++
3	Tannins	+
4	Flavonoids	++
5	Terpenoids	+
6	Steroids	+
7	Glycosides	+
8	Flavanol glycosides	+
9	Cardiac glycosides	-
10	Saponins	+
11	Carbohydrates	++
12	Proteins	++

(+): presence of chemicals, (-): absence of chemicals or not detectable concentration, (+) < (++) < (+++): based on the intensity of characteristic.

of various secondary metabolites like alkaloids, phenols, tannins, steroids, terpenoids, flavonoids, glycosides and saponins. Cardiac glycosides were found to be absent in this extract. The qualitative phytochemical analysis of aqueous extract of *Rhinacanthus nasutus* was performed and the results were show in Table 2.

Phytochemicals are at the heart of phytomedicines and their therapeutic efficacy is directly proportional to their presence. Based on the primary phytochemicals occurrence in the aqueous extract of *Rhinacanthus nasutus* phenolic and flavonoids were quantified in this study. From *Rhinacanthus nasutus* quantified phenolic content about 61.38702% and Flavonoids content 7.882051% (Table 3).

In vitro antioxidant activity

The DPPH method is commonly used to assess the ability of plant extracts and compounds to scavenge the free

Table 3: Quantitative phytochemical analysis of *Rhinacanthus nasutus.*

S.No	Quantitative phytochemical analysis	
	Phytoconstituents	Percentage (%)
1	Phenolics	61.38702
2	Flavonoids	7.882051

Table 4: *In vitro* antioxidant activity.

Sample	Conc (mcg/ml)	Absorbance	Control	% of inhibition	IC 50 % / ml
	10	0.673	0.998	32.56513	
RN	10	0.711	0.998	28.75752	34.30194
	10	0.583	0.998	41.58317	

radicals.[19] DPPH is a type of free radical that changes the colour from violet to yellow when it is reduced by hydrogen or electron donation from the samples. Potent antioxidants of those samples that have the ability to inhibit DPPH.[20] The ability of the aqueous extract of *R. nasutus* to act as donor of hydrogen atoms or electrons in the transition of the DPPH radical into its reduced form was studied in this work. Ascorbic acid and aqueous extract of *Rhinacanthus nasutus* were found to lower DPPH by 28.75 percent and 34.30 percent, respectively (Table 4). Although the aqueous extract of *R. nasutus* showed higher free radical scavenging activity than the reference standard of ascorbic acid.[21] Overall, *Rhinacanthus nasutus* appear to have considerable free radical scavenging activity, which could be beneficial in therapeutic applications.

DISCUSSION

For the selection of a solvent to extract the active ingredients from the matrix, several aspects must be addressed, including selectivity, density, toxicity, volatility, reactivity, physical hazard and miscibility with aqueous media (Prabu and Suriyapraksh, 2012).[22] In present study solvents like water, alcohol, acetone, DMSO, ethyl acetate, chloroform and Hexane were used to find the solubility of *Rhinacanthus nasutus*. Best solubility of *Rhinacanthus nasutus* was observed in Water, alcohol, acetone, DMSO but insolubility observed in other solvents like ethyl acetate, chloroform and Hexane. However solvents like ethyl acetate, chloroform not used for industrial application due to their inherent toxicity and volatility.

Preliminary phytochemical screening of the leaf extracts of *R.nasutus* showed positive results for the presence of secondary metabolites like alkaloids, phenols, tannins, steroids, terpenoids, flavonoids, Carbohydrate, proteins, glycosides and saponins. Cardiac glycosides were found to be absent in this extract. These phytochemical components are known to support bioactive activities in medicinal plants and are thus responsible for the antioxidant activities of the plant extract studied. The free radical scavenging actions most likely due to flavonoids present in the plants. Flavonoids and plant phenolics are two types of chemicals that can act as principal antioxidants and free radical scavengers.[23] In the present study also observed the presence of higher content of phenolic and Flavonoids.

The study of Sharma (2006) also reported that, one of the primary families of phytochemicals, benzopyrone-containing flavonoids, has been linked to a variety of pharmacological actions, including antioxidant and anti-aging characteristics.[24] Flavonoids have also been linked to anti-cancer activity due to their ability to block the estrogen-producing enzyme. Flavonoids are part of the biggest phenolic category, which includes over 600 natural chemicals.[25] Flavonoids can also bind to Gamma-amino butyric acid, a receptors in the nervous system, causing sedative or anxiolytic effects, as well as to regulate the cholinergic nervous system,[26] potentially reducing neurodegenerative processes and impaired cognitive function.[27] From the present work it was observed that, leaf extract of *Rhinacanthus nasutus* contains a reasonably high amount of flavonoids content, which could be used as an effective antioxidant and anti-cancer agent in the future.

Phenolic chemicals are well-known to improving quality and nutritional value by altering colour, taste, scent and flavour, as well as delivering health benefits. They also play a role in plant defence mechanisms to combat reactive oxygen species (ROS) and prevent molecular damage from microbes, insects and herbivores.[28] Phenolic chemicals are employed as nutraceuticals and it can be found in apples, green tea and red wine as secondary metabolites in many medicinal plants. Presence of these phenolic chemicals, which have the ability to limit the generation of ROS by blocking the activation of redox sensitive transcription factors like nuclear-factor, is principally responsible for the plant's antioxidant and reactive oxygen species (ROS) scavenging capacity.[29] Resveratrol the phenolic compound has the ability to halt cell-cycle at various stages in different cancer models. Phenolic compounds have also demonstrated immunomodulatory activity by modulating cytokines and chemokine's.[30]

Tannins have long been used in traditional medicine to treat a variety of ailments. High tannin concentration has been linked to decrease the viral activity. Certain tannins' synergistic actions with various antibiotics have been shown to be effective to fight against antibiotic resistance bacteria. Furthermore, tannin has anti-cancer, anti-mutagenic and tumor-promotion inhibiting properties.[31]

The antioxidant properties of *R. nasutus* leaves were investigated using DPPH as the radical recipient. The aqueous extract of *R. nasutus* showed stronger free radical scavenging activity than the reference standard of ascorbic acid in the current investigation. Many diseases like skin diseases, cancer and pharmacological activity, are caused by free radicals. DPPH is a sensitive method for determining the antioxidant activity of plant extracts because it is stable in the presence of free radicals.[19,32]

The presence of secondary metabolites in aqueous extract of *Rhinacanthus nasutus* supports the anticancer activity. The qualitative and quantitative phytochemical tests established the anticancerous activity and also chemical composition of the aqueous extract which possess bioactive constituents that could contribute towards the treatment of cancer.

CONCLUSION

As a part of physiochemical examination solubility and pH were measured in *Rhinacanthus nasutus*. These metrics can be used in conjunction to preserve purity and quality. The phytochemical analysis of *Rhinacanthus nasutus* aqueous extracts could be useful in determining the quality of the extracts. Except cardiac glycosides, a preliminary qualitative phytochemicals examination of aqueous extract of *Rhinacanthus nasutus* revealed the presence of alkaloids, phenols, saponins, flavonoids, tannins, steroids and terpenoids. The aqueous extract of *Rhinacanthus nasutus* possesses a higher concentration of phenolic compounds and flavonoids, according to quantitative phytochemical study. The results from the *in vitro* antioxidant activity like DPPH assays revealed that the aqueous extract of *Rhinacanthus nasutus* demonstrated the 34.301% of free radical scavenging activity. Presence of these phytochemicals and free radical scavenging activity in the plant sample (*Rhinacanthus nasutus)* may be responsible for their pharmacological activities and therapeutic effect. Their antioxidant action may explain why they are effective in the management and treatment of a variety of disorders and diseases like cancer.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

WHO: World Health Organization; **DMSO:** Dimethyl sulfoxide; **ROS:** Reactive Oxygen Species; **DPPH**: 2,2-diphenyl-1-Picrylhydrazyl.

SUMMARY

The phytochemical analysis of *R. nasutus*, revealed the presence of alkaloids, phenols, saponins, flavonoids, tannins, steroids and terpenoids except cardiac glycosides. It also possesses the good free radical scavenging activity. It will be useful in synthesis and preparation of new drugs in pharmaceutical Industry.

REFERENCES

1. Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. Pharm Res. 2008;25(9):2097-116. doi: 10.1007/s11095-008-9661-9, PMID 18626751.
2. Panghal M, Kaushal V, Yadav JP. *In vitro* antimicrobial activity of ten medicinal plants against clinical isolates of oral cancer cases. Ann Clin Microbiol Antimicrob. 2011;10(21):21. doi: 10.1186/1476-0711-10-21, PMID 21599889.
3. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect. 2001;109(1);Suppl 1:69-75. doi: 10.1289/ehp.01109s169, PMID 11250806.
4. Bidlack WR. Phytochemicals as bioactive agents. Vol. 3. Boca Raton: CRC press Press; 2000:11-20.
5. Robards K, Prenzler PD, Tucker G, Swatsitang P, Glover W. Phenolic compounds and their role in oxidative processes in fruits. Food Chem. 1999;66(4):401-36. doi: 10.1016/S0308-8146(99)00093-X.
6. Govindarajan R, Vijayakumar M, Pushpangadan P. Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. J Ethnopharmacol. 2005;99(2):165-78. doi: 10.1016/j.jep.2005.02.035, PMID 15894123.
7. Tavakoli J, Miar S, Majid Zadehzare MM, Akbari H. Evaluation of effectiveness of herbal medication in cancer care: a review study. Iran J Cancer Prev. 2012;5(3):144-56. PMID 25628834.
8. Gupta AK, Tandon N. Reviews on Indian medicinal plants. New Delhi: Indian Council of Medical Research; 2004.
9. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. Springer Science+Business Media; 1998.
10. Thangaraj P. Proximate composition analysis. Pharmacological Assays of plant-based Natural Products. Springer International Publishing; 2016:21-31.
11. Wagner H, Bladt XS, Gain Z, Suie EM. Plant drug analysis. Germany: Springer Veralag; 1996:360.
12. Mace ME. Histochemical localization of phenols in healthy and diseased banana roots. Physiol Plant. 1963;16(4):915-25. doi: 10.1111/j.1399-3054.1963.tb08367.x.
13. Williamson EM, Okpako DT, Evans FJ. Pharmacological methods in phytotherapy research: Volume 1. Selection, Preparation and Pharmacological Evaluation of Plant Material. John Wiley and Sons Ltd.1996.
14. Raaman N. Phytochemical techniques. New India Publishing Agency, jai Bharat printing press. New Delhi; 2006:19-22.
15. Kokate CK. Practical pharmacognosy. 4th ed. New Delhi, India: Vallabh Prakashan Publication; 1999.
16. Siddhuraju P, Becker K. Studies on antioxidant activities of Mucuna seed (Mucunapruriensvarutilis) extract and various non-protein amino/imino acids through *in vitro* models. J Sci Food Agric. 2003;83(14):1517-24. doi: 10.1002/jsfa.1587.
17. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999;64(4):555-9. doi: 10.1016/S0308-8146(98)00102-2.
18. Blois MS. Antioxidant determinations by the use of a stable free radical. ON Nat. 1958;181(4617):1199-200. doi: 10.1038/1811199a0.
19. Koleva II, Van Beek TA, Linssen JPH, De Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal. 2002;13(1):8-17. doi: 10.1002/pca.611, PMID 11899609.
20. Dehpour AA, Ebrahimzadeh MA, Seyed Fazel N, Seyed Mohammad N. Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. Grasas Aceites. 2009;60(4):405-12. doi: 10.3989/gya.010109.
21. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, Chen S, Corpe C, Dutta A, Dutta SK, Levine M. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am Coll Nutr. 2003;22(1):18-35. doi: 10.1080/07315724.2003.10719272, PMID 12569111.
22. Prabu SL, Suriyapraksh TNK. Extraction of drug from the biological matrix: a review. A applied biological engineering –Principle and practices. p. 480-506. [Online available]; 2012. Available from: http://www.intechopen.com/books/applied –biological engineering –principle-and-practices/ extraction –of- the-drug-from – the – biological-matrix.[accessed on Apr 23, 2015].
23. Polterait O. Antioxidants and free-radical scavengers of Natural Origin. Curr Org Chem. 1997;1:415-40.
24. Sharma DK. Pharmacological properties of flavonoids from plants. J Sci Ind Res. 2006;65:477-84.
25. Kennedy DO, Wightman EL. Herbal Extracts and Phytochemicals: Plant Secondary Metabolites and the Enhancement of Human Brain function. Adv Nutr. 2011;2(1):32-50. doi: 10.3945/an.110.000117.
26. Kim DH, Jeon SJ, Son KH, Jung JW, Lee S, Yoon BH, Lee JJ, Cho YW, Cheong JH, Ko KH, Ryu JH. The ameliorating effect of oroxylin A on scopolamine-induced memory impairment in mice. Neurobiol Learn Mem. 2007;87(4):536-46. doi: 10.1016/j.nlm.2006.11.005, PMID 17196405.
27. Spencer JP. The impact of fruit flavonoids on memory and cognition. Br J Nutr. 2010;104;Suppl 3:S40-7. doi: 10.1017/S0007114510003934. PMID 20955649.
28. Vaya J, Paula AB, Aviram M. Constituents from licorice roots, isolation, structure elucidation and antioxidative capacity toward LDC oxidation. Free Radic Biol Med. 1997;2:302-13.
29. Wahle KWJ, Brown I, Rotondo D, Heys SD. Plant phenolics in the prevention and treatment of cancer. Adv Exp Med Biol. 2010;698:36-51. doi: 10.1007/978-1-4419-7347-4_4, PMID 21520702.
30. Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol. 2006;71(10):1397-421. doi: 10.1016/j.bcp.2006.02.009, PMID 16563357.
31. Okuda T, Ito H. Tannins of Constant Structure in Medicinal and Food Plants—Hydrolyzable Tannins and Polyphenols Related to Tannins. Molecules. 2011;16(3):2191-217. doi: 10.3390/molecules16032191.
32. Suresh PK, Sucheta S, Sudarshana VD, Selvamani P, Latha S . Antioxidant activity in some selected Indian medicinal plants. Afr. J. Biotechnol 2008;7:1826-8.

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