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# PHYTOCHEMICAL SCREENING, EVALUATION OF TOTAL TANNINS, ANTIOXIDANT POTENTIAL AND ANTIBACTERIAL ACTIVITY FROM LEAF AND FLOWER EXTRACTS OF *SPILANTHES CALVA* D.C.

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## ABSTRACT

The present study deals with the phytochemical screening, total tannin content and antibacterial activity from leaf and flower extracts of *Spilanthes calva*. Phytochemical screening of various extracts such as aqueous, ethanol, acetone, chloroform and petroleum ether, revealed the presence of active ingredients such as tannins, saponins, quinones, flavonoids, phenols, cardiac glycosides, terpenoids, steroids and alkaloids in the leaf and flower extracts of *Spilanthes calva*. The leaf and flower extracts were quantitatively estimated for tannin content with tannic acid (TA) as standard. The optimal yield of tannin was found in flower extract 31.5 ± 0.19 mg Tannic Acid Equivalents (TAE) / g) followed by leaf extract 22.8 ± 0.14 mg Tannic acid Equivalents (TAE) / g) of *Spilanthes calva*. The leaf and flower extracts of *Spilanthes calva* were evaluated for antioxidant activities by DPPH (1,1-Diphenyl-2-picryl-hydrazyl) radical scavenging assay. Among five different

solvent extracts of leaf and flower of *Spilanthes calva,* the results recorded that the maximum antioxidant activity was found in the ethanolic leaf (86.41±0.19%) and flower extract (94.87±0.37%) of *Spilanthes calva* followed by other solvent extracts. Different concentrations of ethanolic leaf and flower extracts were tested using the agar disc diffusion method for the antibacterial activity against *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* It was concluded that the powerful antibacterial effect is attributed to the greater amount of tannin compounds in the ethanolic flower extracts of *Spilanthes calva.*

**KEYWORDS:** *Spilanthes calva,* Flower extract, Tannins, Phytochemical analysis, Disc diffusion, Antibacterial activity.

## INTRODUCTION

Plants have been used as a source of medicine throughout the world to preserve human health and are rich source components with a variety of biological activities, including antibacterial activity. Secondary metabolites contained in plants are responsible for their medicinal activities. Approximately 95% of modern drugs have been isolated from traditional medicinal plants.[1] Plants are the richest source of medications for ancient systems of medicine, contemporary medicines, nutraceuticals, dietary supplements, folk remedies, pharmaceutical intermediates and chemical entities for synthesized drugs.[2] Most of the people in rural and urban areas of the world are dependent on the medicinal plants for the treatment of infectious diseases. The Ayurvedic and Unani systems of medicines are widely used by the people of Indian subcontinent. In spite of the recent domination of the synthetic chemistry as a method to discover and produce drugs, the potential of bioactive plants or their extracts to provide new and novel products for disease treatment and prevention is still enormous.[3] Plant derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Dietary phytochemicals are considered as an effective tool to cure body disorder. They play important roles as therapeutic agents in prevention of many diseases.[4] Among the different plant derivatives, secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity.[5]

Tannins have high polyphenolic compounds present in plants, foods and beverages, soluble in water and polar organic solvents. These tannins are classified as hydrolysable and condensed tannins based on their chemical structure and biological activity.[6,7] Both types of tannins are

capable of forming strong complexes with certain type of proteins depressing the rate of their digestion.[8] Tannins can also bind to bacterial enzymes or create indigestible bonds with cell wall carbohydrates, lowering cell wall digestibility.[9,10,11] In recent years, tannins have been investigated to possess high antioxidants[12], free radical scavenging activity[13], antimicrobial[14], gastro protective, and anti-ulcerogenic activities.[15] Moreover, tannins have been investigated as potent inhibitors of lipid peroxidation in heart mitochondria[16] and possess anti-fibrotic effects.[17] Due to these therapeutic properties tannins can be used in the treatment of various diseases to improve human health.

Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and peroxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA.[18] It has been established that oxidative stress is the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, neurodegenerative diseases, immunosuppression and others.[19,20] The screening of plant products for antibacterial activity has shown that the higher plants represent a potential source of novel antibiotic prototypes.[21] There has been an increasing incidence of multiple resistances in human pathogenic microorganism in recent years.[22]

*Spilanthes calva* D.C. (Asteraceae family) is an important medicinal plant with rich source of therapeutic constituents used in Ayurveda. It is commonly known as toothache plant or virus blocker and used as an anti-ageing agent and immunity enhancer.[23] It is an annual herb, grows up to two feet, distributed throughout the peninsular India. The stem is erect or decumbent at base and more or less hairy; flower heads are ovoid and pale yellow in colour. The flowers and leaves have pungent taste and when touched it is accompanied by tingling sensation and numbness.[24,25] Spilanthol, a potent stimulant, sialagogue, and local anesthetic, is produced by the flower head, roots, and entire aerial region.[26] The plant has been used in folk medicine in the treatment of inflammation, toothache, rheumatic fever, skin disease, purgative, diuretic, lithotriptic and dysentery.[27] as fresh vegetable.[28] as well as spice for Japanese appetizer.[29] The flower heads are chewed to relieve toothache and infections of gums, throat and paralysis of the tongue.[30] Due to its high medicinal value, there is an increasing demand in the national and international market.[31] Hence in the present study, the leaf and flower extracts of *Spilanthes calva* were screened for phytochemical constituents, tannins content, antioxidant and antibacterial activity against various human pathogens.

## MATERIALS AND METHODS

### Collection of *Spilanthes calva*

The healthy plants of *Spilanthes calva* leaf and flowers (Fig.1a, b, c) were collected from Kolli Hills, Namakkal district, Tamil Nadu and the plant was authenticated by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre, Sakthi Nagar, West Tambaram, Chennai- 600 045. The collected leaf and flowers were brought to the laboratory processed and shade dried. The shade dried plant samples were maintained at Department of Biotechnology, University of Madras, Guindy Campus, Chennai -600 025.

### Preparation of the Plant Extract

The dried leaf and flowers (15 g each) of *Spilanthes calva* were finely powdered with pestle and mortar and extracted with 150 ml aqueous, ethanol, chloroform, acetone and petroleum ether for 1 minute using an Ultra Turax mixer (13,000 RPM) and soaked overnight at room temperature. The sample was then filtered through Whatmann No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evaporator at 40°C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in refrigerator below 10º C.[32,33]

### Phytochemical Screening from Leaf and Flower Extract of *Spilanthes calva*

The phytochemical screening of leaf and flower extracts of *Spilanthes calva* was assessed by standard method.[34,35] Phytochemical screening was carried out on the leaf and flower extracts using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides**,** coumarins and steroids. General reactions in this analysis revealed the presence or absence of these compounds in the leaf and flower extracts tested.

### Estimation of Total Tannin content from Leaf and Flower Extract of *Spilanthes calva*

Total tannins content from leaf and flower extract of *Spilanthes calva* was estimated according to the method as described by Fagbemi *et al*.[36] The ethanolic extract (1 ml) was mixed with Folin- Ciocalteau's reagent (0.5 ml), followed by the addition of saturated sodium carbonate ($Na_2CO_3$) solution (1 ml) and distilled water (8 ml). The reaction mixture was kept at room temperature for 30 minutes. The supernatant was obtained by centrifugation and absorbance was recorded at 725 nm using UV-Visible Spectrophotometer. Increasing concentrations of standard tannic acid was prepared and the absorbance of various tannic acid

concentrations was plotted for a standard graph. The tannin content was expressed as mg tannic acid equivalent (TAE) per gram of the sample.

**Quantitative Analysis of Free Radical Scavenging Activity of *Spilanthes calva***

The antioxidant activities were quantitatively determined by using 2, 2 diphenyl-1-picryl hydrazyl (DPPH) as a free radical. Leaf and flower extracts of *Spilanthes calva*, 100µl extracts were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control.[37] Subsequently at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517 nm. The antioxidant capacity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates. Free radical scavenging activity was calculated by the prescribed formula: % DPPH radical scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance of control)] x 100.

**Antibacterial Activity of Leaf and Flower Extract of *Spilanthes calva***

The ethanolic leaf and flower extracts of *Spilanthes calva* plant were used for antibacterial study.[38,39] Different concentrations (10mg, 20mg and 30mg /ml) of the concentrated ethanolic leaf and flower extract was tested for its antimicrobial activity against pathogenic bacterial strains such as *Bacillus subtilis, Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa*. The bacterial cultures were grown in Muller Hinton Agar and Muller Hinton Broth (Hi Media).[40]

**Antibacterial Activity Assay**

Antibacterial activity was measured using the standard method of diffusion disc plates on agar.[41] For antimicrobial assay, all bacterial strains were grown in Mueller Hinton Broth Medium (Hi Media) for 24 hours at 37° C and plated on Mueller Hinton Agar (Hi Media) for agar diffusion experiments. Then 0.1ml of each culture of bacteria was spread on agar plate surfaces. Sterile disc (Hi Media, 6mm in diameter) were placed on the agar medium to load 20µl of different concentration (10 -30mg /ml) of ethanolic leaf and flower extracts of *Spilanthes calva* were tested. Inhibition diameters were measured after incubation for 24

hours at 37°C. Blanks of solvent only (processed in the same way), were also tested for antibacterial activity.

## RESULTS AND DISCUSSION

In the present study, phytochemical screening was performed with aqueous, ethanol, chloroform, acetone and petroleum ether of the leaf and flower extracts of *Spilanthes calva.* The phytochemical screening of five different extracts studied, showed that the ethanolic extract of leaf and flower of *Spilanthes calva* were rich in phenols, tannins, saponins, flavonoids, alkaloids and steroids, followed by other extracts (Table 1, 2). Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defence mechanism against predation by many micro-organisms, insects and herbivores. The curative properties of medicinal plants are perhaps due to the presence of various bioactive compounds including alkaloids, flavonoids, phenols, saponins, steroids, etc.[42] Tannin compounds present in many medicinal plants inhibit the growth of many fungi, yeasts, bacteria and viruses.[43] The presence of alkaloids and saponins in leaf extract, the biological function of alkaloids and their derivatives are very important and are used in analgesic, antispasmodic and bactericidal activities.[44] Saponins have properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties, formation of foams in aqueous solutions and haemolytic activity.[45] and traditionally saponins have been extensively used as detergents, as pesticides and molluscicides, in addition to their industrial applications as foaming and surface-active agents and also have beneficial health effects.[46] Plant steroids are known important for their cardiotonic activities and also used in nutrition, herbal medicine and cosmetics. Thus, the preliminary screening tests may be beneficial in the identification of bioactive principles, resulting in the development and discovery of drugs.[47] Furthermore, these assays additionally assist with the quantification and qualitative isolation of pharmacologically active chemical substances.

The result of the present study recorded highest Tannins content in the flower extract of *Spilanthes calva* and the tannins content was expressed as mg tannic acid equivalent (TAE) per gram of the sample. The optimum yield of tannins was found to be 31.5 ± 0.19 mg TAE / g dry weight from flower followed by 22.8 ± 0.14 mg TAE / g dry weight from leaf of *Spilanthes calva* (Table 3). The effect of ethanol on extraction of tannins from *Spilanthes calva* flower extracts was good followed by leaf extract. The results corroborate with the

findings of Singh *et al*.[48] who has reported the maximum yield of Tannins from ethanolic extract of *Artemisia absinthium.* Tannins are the natural polyphenolic compounds which can influence the nutritive value of different food stuffs utilized by human and other animals. Tannins also have large influence on the phytochemical and phytotherapeutical value of medicinal plants. Various methods have been used to increase the extraction efficiency of tannins from different medicinal plants for their use in pharmaceutical field.[49] Ethanol has been found to be the most commonly used solvent for the extraction of tannins rather than other organic solvents.[50] Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes.[51]

Among the five different solvent extracts of *Spilanthes calva* (leaf and flower), the ethanol leaf and flower extract of *Spilanthes calva was* recorded the most effective DPPH radical scavenging activity 86.41 ± 0.19 % (leaf) and 94.87±0.37% (flower) Fig. 2, 3. *Spilanthes calva* flower extract value being very close to synthetic antioxidant (BHT) as positive control (98.6±1.4%). The leaf and flower sample of *Spilanthes calva*, ethanol leaf and flower extracts recorded higher percentage of free radical scavenging activity followed by aqueous, acetone, chloroform and petroleum ether. Scavenging activity for free radicals of DPPH (1,1-Diphenyl-2-picryl hydrazyl) has widely used to evaluate the antioxidant activity of natural products from plant and natural sources. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants.

The data presented in Table 4, indicate that the leaf and flower extracts of *Spilanthes calva* inhibit the growth of some microorganism to various concentration. The concentrations of 10mg/ml - 30mg/ml ethanolic leaf and flower extract displayed antibacterial activity against *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa* and inactivity against *Escherichia coli*. The maximum distinct zone of inhibition has been found at 30mg/ml of ethanolic flower extract of *Spilanthes calva* than leaf extract**.** In both case of leaf and flower extracts, was determined to be inactive against *Escherichia coli* and there is no zone of inhibition has been found in lower concentration (10mg/ml). Similar results were obtained on ethanol extracts from leaves of *Sida acuta and Acalypha wilkesiana* which exhibited antibacterial activity.[52,53] The antimicrobial activities of ethanol extract may be due to the existence of tannins, triterpenoids and flavonoids. Tannins have been known to

form irreversible complexes with proline rich protein resulting in the inhibition of cell wall synthesis.[54]

## CONCLUSION

In conclusion, phytochemical composition, total tannin content, antioxidant activity and antibacterial activity of medicinal plants are very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate an urgent step for screening of plants for secondary metabolites. The present communication attempts to assess the status of phytochemicals, total tannin content, antioxidant activity and antibacterial activity, in the leaf extract of *Spilanthes calva* to improve the health status of people and also to use it in the nutraceuticals products of commercial importance. Thus, from our findings, it is concluded that the ethanolic extracts from dry powdered flower of *Spilanthes calva* had superior level of antimicrobial activity. The stronger antimicrobial effect is attributable to the higher quantity of tannins compound in the ethanolic flower extracts of *Spilanthes calva*.

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## Conflicts of Interest

The authors declare that there is no conflict of interest.

**Table 1: Phytochemical Screening from Leaf Extracts of *Spilanthes calva.***

| Phytochemicals | Leaf Extract of *Spilanthes calva* | | | | |
|---|---|---|---|---|---|
| | Ethanol | Aqueous | Acetone | Chloroform | Petroleum ether |
| Tannins | ++ | + | + | + | + |
| Saponins | ++ | + | - | - | - |
| Quinones | + | - | - | - | - |
| Flavonoids | ++ | + | - | - | - |
| Phenol | + | + | + | + | + |
| Glycosides | - | - | - | - | - |
| Cardiac glycosides | ++ | + | + | - | + |
| Terpenoids | + | + | - | - | - |
| Steroids | + | + | - | - | - |
| Alkaloids | + | - | - | - | - |

++ = indicate strong positive, + = positive, - = negative

**Table 2: Phytochemical Screening from Flower Extracts of *Spilanthes calva***

| Phytochemicals | Flower Extract of *Spilanthes calva* | | | | |
|---|---|---|---|---|---|
| | Ethanol | Aqueous | Acetone | Chloroform | Petroleum ether |
| Tannins | ++ | + | + | + | + |
| Saponins | ++ | ++ | ++ | - | - |
| Quinones | + | - | - | - | - |
| Flavonoids | ++ | + | - | - | - |
| Phenol | ++ | + | + | + | + |
| Glycosides | - | - | - | - | - |
| Cardiac glycosides | ++ | + | - | + | - |
| Terpenoids | + | + | + | - | + |
| Steroids | ++ | + | - | - | - |
| Alkaloids | ++ | + | + | - | - |

++ = indicate strong positive, + = positive, - = negative

**Table 3: Quantitative Estimation of Tannin Content from Leaf and Flower Extract of *Spilanthes calva.***

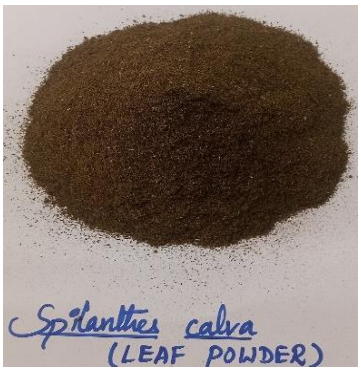
| Extract of *Spilanthes calva* | Tannin Content (mg Tannic Acid Equivalent /g Dry Material) |
|---|---|
| Leaf extract | 22.8 ± 0.14 |
| Flower extract | 31.5 ± 0.19 |

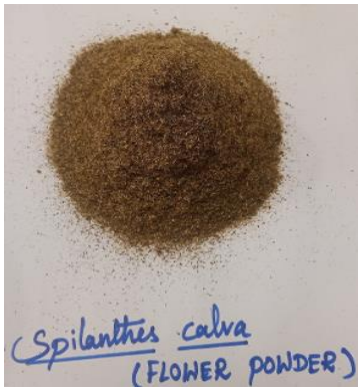
**Table 4: Antibacterial Activity of Leaf and Flower Extract of *Spilanthes calva.***

| Inhibition Zone in Diameter (mm)* | | | |
|---|---|---|---|
| Micro-organisms Tested | Concentrations of Extract | | |
| Ethanolic Leaf Extract | 10mg/ml | 20mg/ml | 30mg/ml |
| *Bacillus subtilis*• | - | 9 | 13 |
| *Bacillus cereus*• | - | - | 11 |
| *Pseudomonas aeruginosa*• | - | - | 9 |
| *Staphylococcus aureus*• | - | - | 8 |
| *Escherichia coli*• | - | - | - |
| Ethanolic Flower Extract | | | |
| *Bacillus subtilis*• | - | 12 | 15 |
| *Bacillus cereus*• | - | 11 | 16 |
| *Pseudomonas aeruginosa*• | - | 12 | 13 |
| *Staphylococcus aureus*• | - | 10 | 14 |
| *Escherichia coli*• | - | - | - |

Note: • This strain was obtained from MTCC, * Includes diameter of disc (6mm); Average three replicates.

**Fig. 1a. Mother Plant of *Spilanthes calva* Collected from Kolli Hills, Namakkal District, Tamil Nadu.**




**1b. *Spilanthes calva* dried leaf biomass and fine leaf powder**

**1c. *Spilanthes calva* dried flower biomass and fine flower powder**

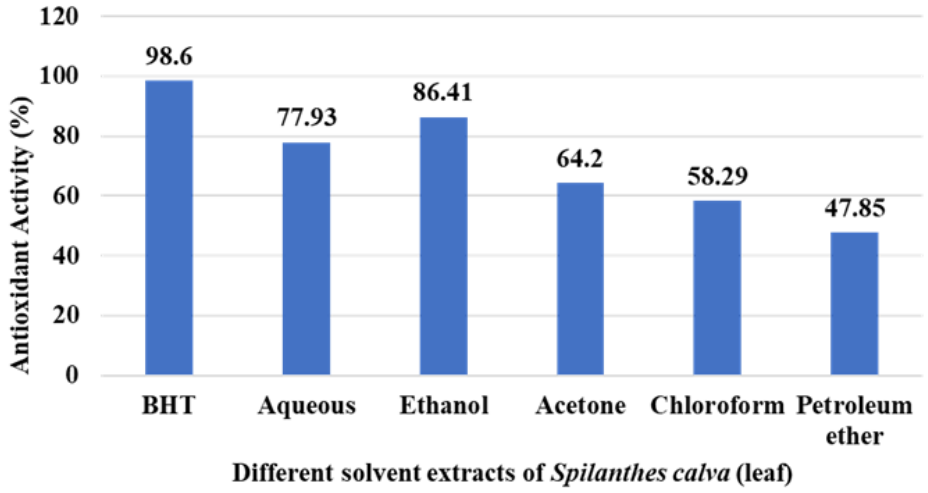



**Fig. 2. Quantitative Analysis of Antioxidant Activity from Leaf Extracts of *Spilanthes calva.***

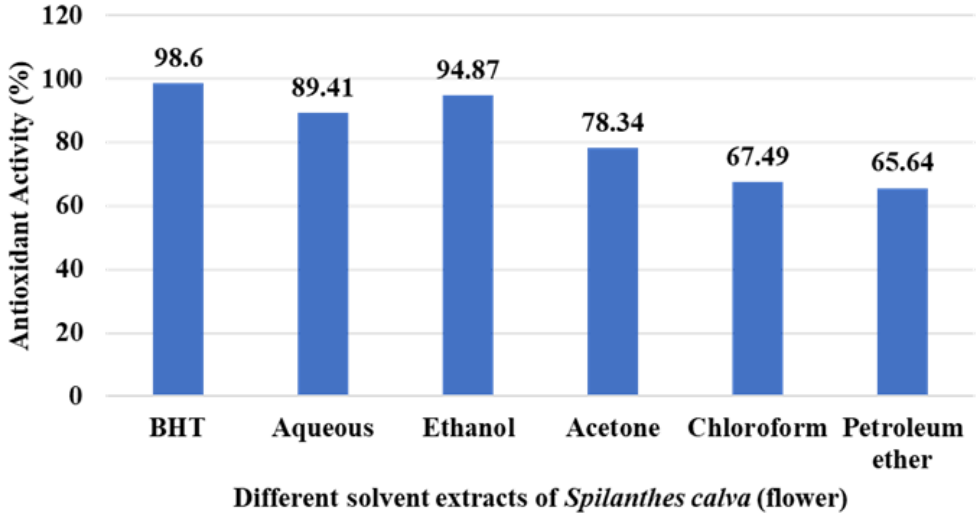



**Fig. 3. Quantitative Analysis of Antioxidant Activity from Flower Extracts of *Spilanthes calva.***

## REFERENCES

1. Rosakutty PJ, Roslin AS. Isolation and characterization of an antimicrobial compound from the traditional medicinal plant Pittosporum tetraspermum Wight & Arn. Int J Med Arom Plants, 2012; 2(1): 141–150.
2. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol, 1999; 86(6): 985–990.
3. Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N, Brinker A, Moreno DA, Ripoll C, Yakoby N, O'Neal JM. Plants and human health in the twenty-first century. Trends in Biotechnology, 2002; 20(12): 522-31.

4. Kareem KT, Kareem SO, Adeyemo OJ, Egberongbe RK. In vitro antimicrobial properties of Bridelia ferruginea on some clinical isolates. Agric Biol J N Am, 2010; 1(3): 416–420.
5. Ahmed AMA, Rahman MS, Anwar MN. Antimicrobial activity of extracts and crude alkaloids of Polyalthia longifolia (Sonn.). Chittagong Univ J Sci., 1999; 23(1): 53–56.
6. Rahman MS, Anwar MN, Chowdhury AZMS. Antibacterial activity of secondary metabolites from Holarrhena antidysenterica stem bark. Bangladesh J Microbiol, 1999; 16(2): 101–105.
7. Haslam EJ. Natural polyphenols (vegetal tannins) as drugs: possible modes of action. J Nat Prod, 1996; 59(2): 205–215.
8. Makkar HPS, Becker K. Do tannins in leaves of trees and shrubs from Africa and Himalayan regions differ in level and activity. Agrofor Syst, 1998; 40(1–3): 59–68.
9. Feeny P. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology, 1970; 51(4): 565–581.
10. Barry TN, Manley RT. The role of condensed tannins in the nutritional value of Lotus pedunculatus for sheep. 2. Quantitative digestion of carbohydrates and proteins. Br. J. Nutr, 1984; 51: 493-504.
11. Barry TN, Manley TR, Duncan SJ. The role of condensed tannins in the nutritional value of Lotus pedunculatus for sheep. 4. Sites of carbohydrate and protein digestion as influenced by dietary reactive tannin concentration. Br J Nutr, 1986; 55(1): 123–137.
12. Reed JD, Soller H, Woodward A. Fodder tree and straw diets for sheep: intake, growth, digestibility and the effects of phenolics on nitrogen utilisation. Anim Feed Sci Technol, 1990; 30(1–2): 39–50.
13. Amarowicz R, Troszyńska A, Baryłko-Pikielna N, Shahidi F. Polyphenolic extracts from legume seeds: correlation between total antioxidant activity, total phenolic content, tannin content and astringency. J Food Lipids, 2004; 11(4): 278–286.
14. Koleckar V, Kubikova K, Rehakova Z, Kuca K, Jun D, Jahodar L, Opletal L. Condensed and hydrolysable tannins as antioxidants influencing health. Mini Rev Med Chem, 2008; 8(5): 436–447.
15. Ho PL, Yung RW, Tsang DN, Que TL, Ho M, Seto WH, Ng TK, Yam WC, Ng WW. Increasing resistance of Streptococcus pneumoniae to fluoroquinolones: results of a Hong Kong multicentre study in 2000. J Antimicrob Chemother, 2001; 48(5): 659–665.
16. Ramirez RO, Roa CC. The gastroprotective effect of tannins extracted from duhat (Syzygium cumini Skeels) bark on HCl/ethanol-induced gastric mucosal injury in Sprague-Dawley rats. Clin Hemorheol Microcirc, 2003; 29(3–4): 253–261.

17. Hong CY, Wang CP, Huang SS, Hsu FL. The inhibitory effect of tannins on lipid peroxidation of rat heart mitochondria. J Pharm Pharmacol, 1995; 47(2): 138- 142.
18. Gutteridge JMC. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem, 1995; 41(12): 1819–1828.
19. Gulcin I, Oktay M, Kufrevioglu OI, Aslan A. Determination of antioxidant activity of lichen Cetraria islandica (L.). Arch J Ethnopharmacol, 2002; 79(3): 325–329.
20. Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in human health: current status and future prospects. J Assoc Physicians India, 2004; 52: 794 –804.
21. Afolayan AJ: Extracts from the shoots of Arctotis artotoides inhibit the growth of bacteria and fungi. Pharm Biol, 2003; 41(1): 22–25.
22. Doss A, Mohammed Mubarack H and Dhanabalan R: Antibacterial activity of tannins from the leaves of Solanum trilobatum Linn. Indian J Sci Technol, 2009; 2(2): 41–43.
23. Chuang HY, Ng LT, Lin LT, Chang JS, Chen JY, Lin TC, Lin CC: Hydrolysable tannins of tropical almond show anti-fibrotic effects in TGF-β1-induced hepatic stellate cells. J Sci Food Agric, 2011; 91(15): 2777–2784.
24. Rai M, Lubraco G. Glomus mosseae enhances the growth of two tropical medicinal plants Withania somnifera and Spilanthes calva. Bol Latinoam Caribe Plant Med Aromat, 2003; 2(4): 56–59.
25. Janarthanam B, Sumathi E. In vitro shoot regeneration from nodal explants of Spilanthes calva. J Trop Med Plants, 2009; 10(2): 209–212.
26. Ghani A. Medicinal plants of Bangladesh with chemical constituents and uses. 2nd ed., Dhaka. Asiatic Society of Bangladesh, 2003; 387.
27. Begum MJ, Nazrul IB, Jasim UC. Essential oil from inflorescence of Spilanthes calva DC. Bangladesh J Bot, 2008; 37(2): 217–218.
28. Mukharya DKK, Ansari AH. Olean-12-en-3-O-β-D-galactopyranosyl-(1→4)-O-α-L-rhamnopyranoside: a new triterpenoidal saponin from the roots of Spilanthes acmella (Murr). Indian J Chem, 1986; 26: 81–87.
29. Tiwari KL, Jadhav SK, Joshi V. An updated review on medicinal herb genus Spilanthes. Chin J Integr Med., 2011; 9(11): 1170–1178.
30. Leng TC, Ping NS, Lim BP, Keng CL. Detection of bioactive compounds from Spilanthes acmella (L.) plants and its various in vitro culture products. J Med Plant Res, 2011; 5(3): 371–378.

31. Rai MK, Acharya D. Screening of some Asteraceous plants for antimycotic activity. Compositae Newsl, 1999; 34: 37–43.
32. Pizzale L, Bortolomeazzi R, Vichi S, Conte LS. Antioxidant activity of sage and oregano extracts related to their phenolic compound content. J Sci Food Agric, 2002; 82(14): 1645–1651.
33. Lu Y, Foo Y. Antioxidant activities of polyphenols from sage (Salvia officinalis). Food Chem, 2001; 75(2): 197–202.
34. Brinda P, Sasikala P, Purushothaman KK. Pharmacognostic studies of Merugan kizhangu. Bull Med Eth Bot Res, 1981; 3: 84 –96.
35. Siddiqui AA, Ali M. Practical pharmaceutical chemistry: 1st ed. New Delhi: CBS Publishers and Distributors, 1997; 126–131.
36. Fagbemi TN, Oshodi AA, Ipinmoroti KO. Processing effects on some anti-nutritional factors and in vitro multi enzyme protein digestibility (IVPD) of three tropical seeds: breadnut (Artocarpus altilis), cashew nut (Anacardium occidentale) and fluted pumpkin (Telfairia occidentalis). Pak J Nutr, 2005; 4(4): 250–256.
37. Lee SE, Hwang, HJ, Ha JS. Screening of medicinal plant extracts for Antioxidant activity. Life Sci, 2003; 73: 167-179.
38. Ozkan G, Sagdic O, Baydar NG, Baydar H. Antioxidant and antibacterial activities of Rosa damascena flower extracts. Food Sci Technol Int, 2004; 10(4): 277–281.
39. Janarthanam B, Sumathi E. Antimicrobial activity of Gymnema sylvestre leaf and callus extracts. J Trop Med Plants, 2010; 11(2): 143–147.
40. Lopez A, Hudson JP, Towers GHN. Antiviral and antimicrobial activities of Colombian medicinal plants. J Ethnopharmacol, 2001; 77(2-3): 189–196.
41. Erturk O, Kati H, Yayli N, Demürbaú Z. Antimicrobial properties of Silene multifida (Adams) Rohrb. Plant extracts. Turk J Biol, 2006; 30(1): 17–21.
42. Britto JD, Sebastian SR. Biosynthesis of silver nano particles and its antibacterial activity against human pathogens. Int J Pharm Sci., 2011; 5: 257-9.
43. Chung KT, Wong TY, Huang YW, Lin Y. Tannins and human health: a review. Crit Rev Food Sci Nutr, 1998; 38(6): 421–464.
44. Stary F. The natural guide to medicinal herbs and plants. London: Tiger Books International, 1998; 12-16.
45. Sodipo OA, Akiniyi JA, Ogunbamosu JU. Studies on certain characteristics of extracts of bark of Pausinystalia macruceras (K. Schum) Pierre ex Beille. Glob J Pure Appl Sci, 2000; 6(1): 83- 87.

46. Shi J, Kakuda Y, Yeung D. Antioxidative properties of lycopene and other carotenoids from tomatoes: synergistic effects. BioFactors, 2004; 21(1-4): 203-210.
47. Doss A, Mohammed MH, Dhanabalan R. Antibacterial Activity of Tannins from the leaves of Solanum trilobatum Linn. Indian J. Sci. Technol, 2009; 2(2): 41-43.
48. Singh R, Kumar P, Singh VG. Total phenolic, flavonoid, and tannin contents in different extracts of Artemisia absinthium. J Intercult Ethnopharmacol, 2012; 1(2): 101–104.
49. Cobzac S, Moldovan M, Olah NK, Bobos L, Surducan E. Tannin extraction efficiency from Rubus idaeus, Cydonia oblonga and Rumex acetosa using different extraction techniques and spectrophotometric quantification. Acta Univ Cibiniensis Ser F Chem, 2005; 8(2): 55–59.
50. Doshi H, Satodiya H, Thakur MH, Parabia F, Khan A. Phytochemical screening and biological activity of Calotropis procera (Ait.) R. Br. (Asclepiadaceae) against selected bacteria and Anopheles stephensi larvae. Int J Plant Res, 2011; 1(1): 29–33.
51. Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Rice-Evans C. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Arch Biochem Biophys, 1995; 322(2): 339–346.
52. Oboh IE, Akerele JO, Obasuyi O. Antimicrobial activity of the ethanol extract of the aerial parts of Sida acuta Burm.f. (Malvaceae). Trop J Pharm Res, 2007; 6(4): 809- 813.
53. Gotep JG, Agada GOA, Gbise DS, Chollom S. Antibacterial activity of ethanolic extract of Acalypha wilkesiana leaves growing in Jos, Plateau State. Nigeria. Malays J Microbiol, 2010; 6(2): 69–74.
54. Mamtha B, Kavitha K, Srinivasan KK, Shivananda PG. An in vitro study of the effect of Centella asiatica (Indian pennywort) on enteric pathogens. Indian J Pharmacol, 2004; 36(1): 41- 44.