Research Article

A Comparitive Study of Antioxidant Properties and Phytochemical Composition of *Trapa bispinosa, Trigonella foenum-graecum, Syzygium cumini* and *Betula utilis*

Saumya Singh, Himani Badoni, Vinay Kumar, Ayush Madan, Promila Sharma, Syed Mohsin Waheed*
Department of Biotechnology, Graphic Era University, 566/6 Bell Road, Clement Town, Dehradun, Uttarakhand, India.
***Corresponding author's E-mail:** syedmohsinwaheed@yahoo.com

Accepted on: 15-04-2016; **Finalized on:** 31-05-2016.

ABSTRACT

Current work was undertaken with the aim of exploring the antioxidant properties of various plant extracts and their potential therapeutic use. Methanolic extract of 4 plants namely *Trapa bispinosa, Trigonella foenum-graecum, Syzygium cumini* and *Betula utilis* were investigated for their phytochemical composition and antioxidant properties. *Trigonella foenum-graecum* extract was found to have the highest antioxidant activity demonstrated by DPPH inhibition assay and the extracts of all the four plants have antioxidant properties. Besides, the extracts of these plants were tested for various classes of phytoconstituents present in these plants to determine the phytochemical composition. All the four plant extracts have antioxidant property and at least one compound from the class of phytocompounds like alkaloids, flavonoids reducing sugars and tannins as a constituent. The present study is a part of larger project that would explore for the therapeutic potential of these extracts in various diseases resulting from oxidative stress and especially of neuronal origin that would modulate a potential target in neurons and/or brain. The extracts showing best antioxidant properties will potentially have good therapeutic properties for the diverse kinds of diseases resulting from oxidative stress.

Keywords: *Trapa bispinosa, Trigonella foenum-graecum, Syzygium cumini, Betula utilis,* antioxidant, phytoconstituents, oxidative stress.

INTRODUCTION

Traditional medicine has a long history since ancient times. It is a bundle of knowledge, proficiencies and practices based on theories, view points and experiences native to different cultures, and inexplicable and vague at times, nevertheless useful in protection of health. Traditional medicine is the synthesis of beneficial experience of generations of physicians practicing indigenous systems of medicine. The names alternative/complementary/non-conventional/indigenous medicine are used interchangeably as opposed to conventional medicine in some countries. India is one of the 12 mega biodiversity centers having 45, 000 species of plant and its biodiversity is unmatched because it has 16 different agroclimatic regions including 10 vegetative zones and 15 biotic regions[1]. The rich floral diversity makes India a major practitioner of traditional medicines like Ayurveda, Unani, Siddha and Homeopathy. The traditional medicine preparations include therapeutic plants, minerals and organic matters etc. Even therapeutic drugs used in conventional medicine are derived from plants thus making them directly or indirectly dependent upon the traditional medicine.

Trapa bispinosa, Trigonella foenum-graecum, Syzygium cumini, and Betula utilis plants were chosen because of their immense medicinal value in traditional medicine. *Trapa bispinosa* is an aquatic floating herb belonging to the family trapaceae. It grows throughout Africa and Asia in lakes and ponds and is often cultivated for its edible fruit. The therapeutic value of the whole herb and fruit has long been documented in conventional medicine as a cure for various ailments. The entire herb has been reported for hepatoprotective, antimicrobial, antibacterial, antitumor, antioxidant and free radical scavenging activities[13].

Trigonella foenum-graecum Linn. (Fenugreek) is an annual herb belonging to Leguminosae family, grown in India, Egypt, and Middle Eastern region. It is one of the oldest medicinal plants, which is commonly used in traditional medicine. The plant is reported to have anti-hyperglycemic effect and used as antidiabetic agent. It has diuretic, uterine & cardio tonic effects, hypotensive, hypolipidemic, hypoglycemic, hyperinsulinemic, antidiabetic activities, and also antinociceptive and anti-inflammatory action[14].

Syzygium cumini (Myrtaceae) also known as *Syzygium jambolanum* and *Eugenia cumini*, commonly known as Jamun. *S. cumini* is an important medicinal plant in various traditional systems of medicine. It is effective in the treatment of diabetes mellitus, inflammation, ulcers and diarrhea and preclinical studies show that it possesses chemopreventive, radioprotective and antineoplastic properties (ref). The plant is rich in phytocompounds containing namely malic acid, oxalic acid, gallic acid, betulinic acid, quercetin, myricetin, myricitin, myricetin. Myricetin works as a strong antioxidant and quercetin shows protective effect against neurological disorders[25].

Betula utilis D. Don (Betulaceae) known as Himalayan birch is a moderate-sized tree that grows up to 20 m in height. The bark is shining, reddish-white or white, with

white horizontal smooth, lenticels. *Betula utilis* shows various pharmacological activities like antimicrobial, anti-inflammatory, anticancer, antioxidant and anti-HIV activities. The plant possesses various alkaloids having diverse therapeutic effects[24].

Various drugs have been used and cited in various treatises of traditional medicines. The plants *Syzygium cumini* (SC), *Trigonella foenum-graecum* (TF) *Trapa bispinosa*(TB) *Betula utilis* (BU) were selected to explore their antioxidant properties and there by their therapeutic activity against various kinds of diseases involving oxidative stress. Biological system during the course of respiration, physiologically or pathophysiologically, produces harmful intermediates called reactive oxygen species (ROS). Excess ROS in the body can lead to damage of proteins, lipids, and DNA, resulting in so-called oxidative stress. The excess production of reactive oxygen species results in oxidative stress, causing cellular damage, because ROS can react with and damage cellular macromolecules, like DNA, proteins and lipids[6-8]. Oxidative stress is the cause for many age-related neurodegenerative diseases. Thus, the phytoconstituents having antioxidant activity can mitigate the effects of oxidative stress and therefore can be used for therapeutic purpose in case of diseases occurring through oxidative stress.

MATERIALS AND METHODS

Material

Plant parts, methanol, DPPH, Fehling's solution A and Fehling's solution B, ethanol, distilled water, HCl, chloroform, conc. H_2SO_4, ammonia solution, picric acid, hexane, alpha naphthol, NaOH , $CuSO_4$, ninhydrin solution, acetic acid anhydride, ferric chloride.

Methodology

Plant collection

The plant *Syzygium cumini* (leaves), *Trigonella foenum-graecum* (seed) collected from Dehradun, Uttarakhand, India, and *Trapa bispinosa* (fruit) collected from Roorkee, *Betula utilis* (bark) collected from Bhojbasa, Uttarkashi district of Uttarakhand India.

Plant authentication

The plants *Trapa bispinosa, Trigonella foenum-graecum, Syzygium cumini* and *Betula utilis were authenticated from* Botanical Survey of India with accession numbers (*Trigonella foenum graecum* Acc No. 114996, *Trapa bispinosa* Acc No. 114995, *Syzygium cumini* Acc. No. 114993, *Betula utilis* Acc. No. 114994).

Preparation of methanolic extracts

TB, TF, SC and BU plant parts were dried under shade and powdered in laboratory. The prepared powder forms were soaked overnight in methanol prior to extract preparation in Soxhlet apparatus.

The powders (200 grams) of TF, SC, BU and TB put in 7:3 methanol water mixtures for boiling using Soxhlet apparatus for 8 cycles at 68°C for ~8 hours with intermittent shaking. The prepared extract was filtered and evaporated by rotary evaporator at 60°C. The dried extract powder was kept in a glass container in a refrigerator and used subsequently for the preliminary screening of phytochemicals and experimental studies.

Preparation of aqueous extracts

TF, SC, BU and TB powders (200 grams) were boiled in distilled water (1000 ml) for 15-20 minutes, then left overnight at room temperature and filtered next morning. The filtrate was evaporated to concentrate in hot air oven at 80°C and concentrate was stored in refrigerator. The concentrated extracts were used for preliminary screening of phytochemicals and experimental studies.

Determination of antioxidant activity by DPPH radical scavenging method

The DPPH radical scavenging capacity of each extract was determined using method of Miliauskas[9,10]. DPPH shows maximum absorption at 515 nm wavelength, the color disappears by reduction of antioxidant compound. The DPPH solution was prepared in methanol (6×10^{-5} M), and 2 ml of this solution was mixed with 100 µl of Methanolic extracts using different concentrations (20, 40, 80µg/ml). The samples were incubated for 30 min at 37 °C, and then absorbance was measured at 515 nm (*A*E). A blank sample was prepared with 100 µl of methanol and 2 ml of DPPH solution and absorbance was measured (*A*B). Standard was prepared using 1% ascorbic acid and serially diluted to 3.125% of original. Ascorbic acid matched concentration used as control. The experiment was carried out in triplicate and each experiment was repeated three times. % inhibition was calculated using the following formula:

(% inhibition) = [(*A*B-*A*E)/*A*B] *100

Where *A*B absorbance of the blank sample and *A*E is absorbance of the plant extract.

RESULTS

Phytochemical qualitative characterization

Table 1: Preliminary photochemical Investigation[11]

S. No	Test	Observation	Inference
1.	**Test for carbohydrates** Molisch's test (general test): to 2-3 ml extract, add few drops of alpha naphthol solution in alcohol. Shake & add concentrated H_2SO_4 from the side of a test tube.	Violet ring is formed at the junction of two liquids.	Carbohydrates are present.
2.	**Test for proteins** Biuret test (general test): To 3 ml extract add 4% NaOH & few drops of 1% $CuSO_4$ solution.	No violet or pink colour appears.	Proteins/peptides are absent.
3.	**Test for amino acids** Ninhydrin test (general test): heat 3 ml extract and 3 drops of 5% ninhydrin solution in boiling water bath for 10 min.	Purple or bluish colour appears.	Amino acids are present.
4.	**Test for fats and oils:** Place a small amount of extract on glass slide. Make a smear. Add a drop of Sudan Red III reagent. After 2 min. wash with 50% alcohol mount in glycerin observe under microscope.	No oil globules are observed.	Fats and oils are absent.
5.	**Test for steroid:** Liebaermann-Burchard reaction: mix 2 ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops of conc. H_2SO_4 from the side of the test tube.	Chloroform layer appears red and acid layer shows greenish yellow fluorescence.	Steroids are present.
6.	**Test for cardiac glycosides:** (Keller- Killiani test): to 2 ml extract, add glacial acetic acid, 1 drop 5% $FeCl_3$ and conc. H_2SO_4.	Reddish brown colour appears at the junction of two liquid layers and upper layer appears bluish green.	Cardiac glycosides are present.
7.	**Test for saponin glycosides** Foam test: shake the drug extract or dry powder vigorously with water.	Persistent foam observed.	Saponinglycosides/saponins are present.
8.	**Test for alkaloids** Wagner's test: 2-3 ml filtrate with few drops of Wagner's reagent.	Reddish brown ppt.	Alkaloids are present.
9.	**Test for tannins** Add few drops of 5% $FeCl_3$ solution in extracts.	Deep blue-black colour.	Tannins are present
10.	**Test for flavonoids** Ferric chloride test – test solution treated with few drops of ferric chloride solution	Blackish red colour formation occurs.	Flavonoids are present

Preparation of extracts

Table 2: Percentage yield of plant extracts

Extract	TB (A)	TB (M)	TF (A)	TF (M)	SC (A)	SC (M)	BU (A)	BU (M)
Percentage yield (gms)	26gm (13 %)	27 gm (13.5 %)	30 gm (15 %)	29.8 gm (14.9 %)	23.92gm (11.96 %)	27.2gm (13.6%)	24 gm (12 %)	25.8 gm (12.9 %)

Table

Extract constituents	TB (A)	TB (M)	TF (A)	TF (M)	SC (A)	SC (M)	BU (A)	BU (M)
Carbohydrates	+	+	+	+	+	+	+	+
Proteins	+	-	+	+	+	+	+	+
Amino acids	+	+	+	+	+	+	+	+
Fats and oils	-	-	-	-	-	-	-	-
Steroids	-	-	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	-	-
Alkaloides	+	+	+	+	+	+	+	+
Tannin	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+

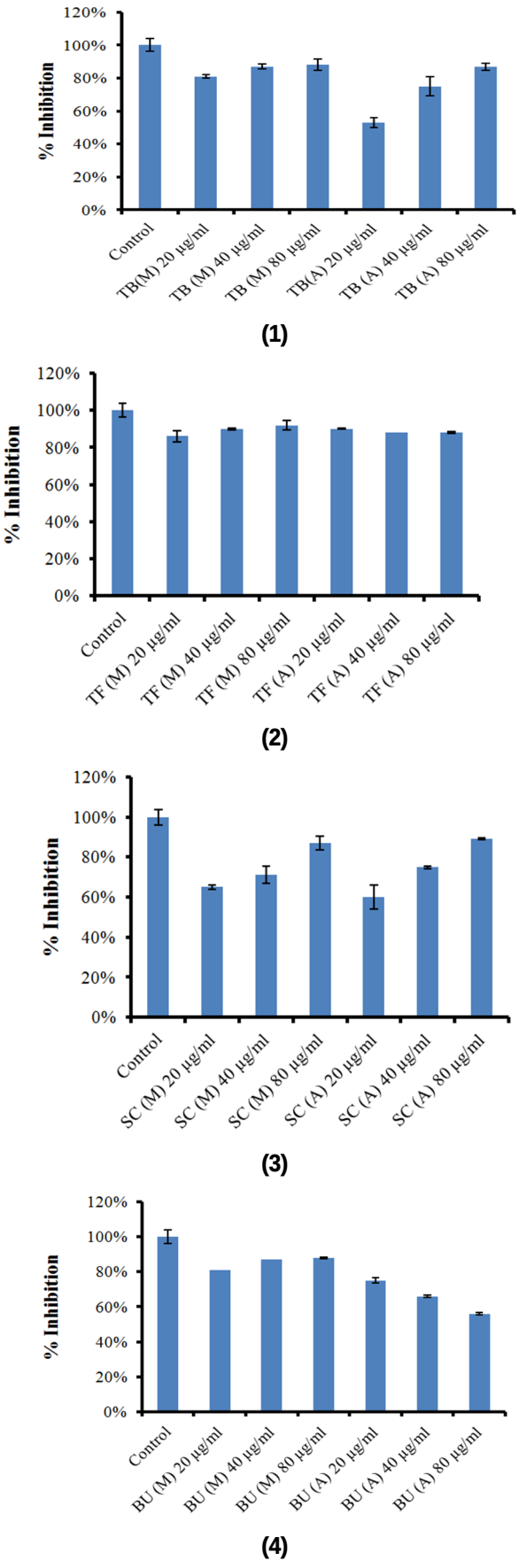

Figure: DPPH assay (1) DPPH radical scavenging activities of *Trigonella foenum-graecum* extract extract, **(2)** DPPH radical scavenging activities of *Trapa Bispinosa* extract, **(3)** DPPH radical scavenging activities of *Syzygium cumini*, **(4)** DPPH radical scavenging activities of *Betula utilis* extract.

A- Aqueous

M-Methanolic

The results of our laboratory prepared extracts from the four medicinal plants are presented in table 2. The percentage yield of the extracts were 26 gm **(**13 %) and 27 gm (13.5 %) for *Trapa bispinosa*, 30 gm (15 %) and 29.8 gm (14.9 %) for *Trigonella foenum-graecum*, 23.92 gm (11.6 %) and 27.2 gm (13.6 %) for *Syzygium cumini* and 24 gm (12 %) and 25.8 gm (12.9) for *Betula utilis* aqueous and methanolic extracts respectively.

Plant authentication

The plants *Trapa bispinosa, Trigonella foenum-graecum, Syzygium cumini* and *Betula utilis was authenticated from* Botanical Servay of India with accession number (*Trigonella foenum graecum* Acc No. 114996, *Trapa bispinosa* Acc No. 114995, *Syzygium cumini* Acc. No. 114993, *Betula utilis* Acc. No. 114994).

Preliminary photochemical Investigation

Important medicinal phytochemicals such as reducing sugars, flavonoids, alkaloids, tannins, proteins, amino acids, steroids and cardiac glycosides were tested. The result of the phytochemical analysis shows that the all four plants are rich in at least one of proteins, amino acid, steroid, cardiaglycoside, tannin, alkaloids, flavonoids reducing sugars and tannins.

DPPH assay

DPPH radical scavenging activities of plant extracts varied from 56% to 92%. *Trigonella foenum-graecum* extract showed the highest antioxidant activity of 92%, 90% and 86% inhibition in methanolic extract and in aqueous extract inhibition of 90%, 88% and 88% of DPPH was recorded. In *Trapa bispinosa* plant methanolic extract DPPH inhibition of 88%, 87% and 81%, and in aqueous extract 87%, 75% and 53% was observed. *Syzygium cumini* methanolic extract showed DPPH inhibition of 87 %, 71% and 65% and in aqueous extract inhibition observed was 89%, 75% and 60%. Inhibition in *Betula utilis* methanolic extract was 88%, 87% and 81%; and in aqueous extract DPPH inhibition was 56%, 66% and 75% inhibition at 80, 40 and 20µg/ml concentrations respectively.

DISCUSSION

The extracts of four plants that were part of this study gave yields in the range of approximately 12-15% (Table 1) and are rich in various classes of phytocompounds like alkaloids, tannins, flavonoids, cardiac glycosides, steroids, amino acids and carbohydrates (Table 2). All these classes of phytoconstituents exert diverse effects on physiology depending upon their composition.

The plants BU, SC, TF and TB have been used to ameliorate various diseases like constipation, inflammation in gastritis, arthritis, alcohol induced liver toxicity, heart diseases, cancer, obesity, and are known to be hypoglycemic, neuroprotective and beneficial for liver function, blood lipids, proapoptosis, cardiovascular health. In addition their phytoconstituents act as cytotoxic agents against malignant brain-tumors but their role in curing diseases of neuronal origin is explored to lesser extent. Most studies provide only the superficial information, lacking depth and targets to cure the diseases. Traditional awareness of plants for medicine is based on personal experiences and the knowledge handed down from one generation to next generations mostly by word of mouth or also in the form written treatise or manuscripts in various Indian vernacular languages. By 19th century active principles of medicinal plants were isolated based on such knowledge base and discovery of quinine from Cinchona bark was the first active principle isolated and characterized[4]. Extract of TF contains caparine, fenugrikine, gentianineyamogenin, gitogenin, neotigogens 4-hydroxyisoleucine. whereas methanolic extract TF is composed of caparine, trignollin, fenugrikine, gentianine, tigogenin, neotigogens, 4-hydroxyisoleucine and the aqueous extract of TB contains pyridoxine, thiamine, nicotinic acid, D amylase, pantothenic acid, phosphorylase, 2β,3α,23-trihydroxyurs-12-en-28-oic acid whereas methanolic extract composition is pyridoxine, thiamine, nicotinic acid, D amylase, pantothenic acid, phosphorylase, 2β,3α,23-trihydroxyurs-12-en-28-oic acid (Pubchem).

Both BU and SC plants are known for their medicinal value for curing various pathophysiological conditions. The phytoconstituents of BU are betulin, lupeol, oleanolic acid, acetyloheanolic acid, betulitc acid, lupenone, sitosterol, methyle betulonate, methyl betulate and karachic acid. BU is known for its antiseptic, proapatotic properties. Oleanic acid have anti-inflammatory and anti-tumor properties and it prevents from cerebral ischemia[20,21]. Betulinic acid is well-known as a development inhibitor of human melanoma, neuroectodermal and malignant tumor cells and it was also reported to stimulate apoptosis in these cells. Betulinic acid acts as an anticancer agent operating through different mechanisms and has been reported to activate apoptotic pathways in cancer cells[22]. The plant SC has been previously reported for the gastroprotective and anti-ulcerogenic, anti-inflammatory, hypoglycemic, hypolipidemic, antianaemic, antioxidant properties. SC works as strong antioxidant and thus it has oxygen radical scavenging capacity. The SC contains phytochemicals namely malic acid, oxalic acid, gallic acid, betulic acid, quercetin, myricetin, myricitin. Myricetin works as strong antioxidant and quercetin shows protective effect in neurological disorders[23]. Owing to their antioxidant rich phytochemical nature these plants have immense therapeutic potential to fight against various types of diseases of diverse origin and nature viz., infectious diseases of bacterial, fungal and viral nature, life style diseases like diabetes and hypertension, diseases of neuronal origin like, epilepsy and dementia, neurodegenerative diseases like AD and PD, besides various forms and types of cancers.

This investigation supports that four plants are promising source of natural antioxidants. Antioxidant properties differ significantly among the four selected plant extracts depending upon the content and composition of the phytoconstituents. Among these plant extracts, *Trigonella foenum-graecum* and *Trapa bispinosa* showed very strong antioxidant properties. The plant BU shows less antioxidant activity in inverse dose dependant manner in contrast with other three plants taken in this study, but that does not undermine its therapeutic value since it has strong proapoptotic nature due to the presence of betulinic acid. Our results are in conformation with a previous study on *B. utilis*[24].

REFERENCES

1. Chopra R.N., Nayar S.L. and Chopra I.C. In Glossary of Indian medicinal plants, *Council of Scientific and Industrial Research*, *New Delhi*, 1, 1956, 197.
2. *WHO traditional medicine strategy 2002–2005*, Geneva: WHO; 2002.
3. Willcox ML, Bodeker G. Traditional herbal medicines for malaria, British Medical Journal, 329, 2004, 1156-9.
4. Phillipson J D. Phytochemistry and medicinal plants, Phytochemistry, 56, 2001, 237-243.
5. Swerdlow Joel L. Nature's Medicine: Plants That Heal, National Geographic Society Publication, 2000.
6. Cerutti PA, Trump BF Inflammation and oxidative stress in carcinogenesis, Cancer Cells, 3(1), 1991, 1-7.
7. Ames BN, Shigenaga MK. Oxidants are a major contributor to aging. Annals of the New York Academy of Sciences, 663, 1992, 85–96.
8. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and degenerative diseases of aging. Proceedings of the National Academy of Sciences, 90, 1993, 7915–22.
9. Miliauskas, G, Venskutonis, PR, Van Beek, TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts, Food Chemistry, 85, 2004, 231–237.
10. Brand-Williams, W Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology, 28, 1995, 25–30.
11. Harborne JB. Phytochemical Methods. New Delhi: Springer (India) Pvt. Ltd, 2005, 17.
12. Kim JH, Park SH, Kim YW, Ha JM, Bae SS, Lee GS. The Traditional Herbal Medicine, Dangkwisoo-San, Prevents Cerebral Ischemic Injury through Nitric Oxide-Dependent Mechanisms, Evidence *Based* Complementary Alternative Medicine 2011. Journal of_Ethnopharmacol, 140(1), 2012, 107–116.
13. Khosla P, Gupta DD, Nagpal RK. Effect of Trigonella foenum graecum (Fenugreek) on blood Glucose in normal and

diabetic rats. Indian Journal of Physiol Pharmacol, 39(2), 1995, 173-4.

14. Das PK, Bhattacharya S, Pandey JN, and Biswas M. Antidiabetic activity of *Trapa natans* fruit peel extract against streptozotocin induced diabetic rats. Global Journal of Pharmacol, 5(3), 2010, 186-190.
15. Dakshinamurtia K, Sharma SK, Jonathan A, Geiger D. Neuroprotective actions of pyridoxine. Biochim Biophys Acta, 1647, 2003, 225-229.
16. Vyawahare NS, and Ambikar DB. Evaluation of neuropharmacological activity of hydroalcoholic extract of fruits of *Trapa bispinosa* in laboratory animals. International Journal Pharma Science, 22, 2010, 32-35.
17. Fulda S, Friesen C, Los M, Scaffidi C, Mier W, Benedict M, Nunez G, Krammer PH, Peter ME, Debatin KM. Betulinic acid triggers CD95 (APO 1/Fas)-and p53-independent apoptosis via activation of caspases in neuroectodermal tumors. Cancer Research, 57, 1997, 4956–4964.
18. Fulda S, Jeremias I, Steiner HH, Pietsch T, Debetulinic acidtin KM. Betulinic acid: a new cytotoxic agent against malignant brain-tumor cells. International journal of cancer, 82, 1999, 435–41.
19. Kumar A, Ilavarasan R, Jayachandran T. Antiinflammatory activity of *Syzygium cumini* seed. African Journal of Biotechnology, 7, 2008, 1932-40.
20. Laura Caltana, María Luisa Nieto, and Alicia Brusco Oleanolic acid: a promising neuroprotective agent for cerebral ischemia Neural Regenration Research, 10(4), 2015, 540–541.
21. Caltana L, Rutolo D, Nieto ML, Brusco A. Further evidence for the neuroprotective role of oleanolic acid in a model of focal brain hypoxia in rats. Neurochemestry International, 79, 2014, 79-87.
22. Fisher DE. Apoptosis in cancer therapy: crossing the threshold, Cell, 78(4), 1994, 539–542.
23. Choi DH, Li C, Choi JS. Effects of myricetin, an antioxidant, on the pharmacokinetics of losartan and its active metabolite, EXP-3174, in rats: possible role of cytochrome P450 3A4, cytochrome P450 2C9 and P-glycoprotein inhibition by myricetin. Journal of Pharmacy and Pharmacology, 62(7), 2010, 908-14.
24. Kumaraswamy M. V. and Satish S. Free radical scavenging activity and lipoxygenase inhibition of Woodfordia fructicosa Kurz and Betula utilis Wall, African Journal of Biotechnology, 7(12), 2008, 2013-2016.
25. Aqil F, Gupta A, Munagala R, Jeyabalan J, Kausar H, Singh IP, Gupta RC. Antioxidant and antiproliferative activities of anthocyanin/ ellagitannin-enriched extracts from Syzygiumcumini L. ('jamun', the Indian Blackberry) Nutr Cancer, 64(3), 2012, 428–438.

Source of Support: Nil, **Conflict of Interest:** None.