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Phytochemical composition and DNA barcoding of *Bauhinia scandens* leaf extract and its *in vitro* assessment of antioxidant and anti-inflammatory potential

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Abstract

Plants belonging to the genus *Bauhinia* are gaining more attention in recent years due to the presence of novel phyto compounds. Out of the several species in this genus, *Bauhinia scandens* is the least explored and thus only very few reports are available with regard to it. Thus, the present investigation focussed on the phytochemical composition of ethanolic extract of *B. scandens* leaves and the *in vitro* assessment of antioxidant as well as anti-inflammatory potential of the extract. GC-MS analysis of the ethanol extract revealed the presence of seven major compounds and Benzoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester indicated the highest peak value in the extract (peak area: 22.09 %). The presence of flavonoids like vitexins, isoflavones, quercetins, rutins, syringate, gallocatechins, kaempferols, alkaloids, glucosides through multiple gas chromatographic mass spectral analyses demonstrated their pharmacological importance. The phosphomolybdate method was used to analyse the total antioxidant activity of the extract. The EC_{50} value obtained for antioxidant capacity for the ethanol extract (23.93±0.12 µg/mL) and was comparable with that of the standard (ascorbic acid: 24.73±0.09 µg/mL. The IC_{50} values of scavenging DPPH radicals for the extract and standard (ascorbic acid) were 39.64±0.02 µg/mL and 37.40 ±0.50 µg/mL, respectively. Strong antioxidant activity of the extract could be attributable to the presence of phenolic compounds. The IC50 values for the extract and standard were 32.22±0.50 µg/mL and 36 ±0.20 µg/mL. Thus this study proved *Bauhinia scandens* as candidate species in pharmaceutical sector.

Keywords: Antioxidant activity, Anti-inflammatory activity, *Bauhinia scandens* leaves, DNA barcoding, Gas chromatography mass spectroscopy

1. Introduction

The Bauhinia genus contains around 300 species that are commonly referred to as 'cow's paw' or 'cow's hoof' due to the shape of their leaves. The genus Bauhinia is comparatively large and consist of trees, climbers and shrubs and are distributed in a broad range of geographic locations (Valdir et al. 2009; Sun et al. 2015). Their leaves, stem and bark have

been used recurrently in folk medicine as an antidote for diabetes, pain and inflammatory processes (Cavalcanti and Favoreto 2005; Singh et al. 2016).

The biological properties of various *Bauhinia* phyto preparations and crude metabolites have been examined *in vivo* and *in vitro* models and these results concluded that these medicinal properties are mainly due to the presence of compounds called flavonoids, terpenes, steroids, aromatic or organic acids, quinones, lactones and alkaloids etc (Ahmed et al. 2012). Anti-diabetic (Miyake et al. 1986; Chaudhari et al. 2013), antimicrobial (Achenbach et al. 1988; Pandey 2015), anti-inflammatory, antioxidant (Aderogba et al. 2007; Rong et al. 2013), antimalarial (Kittakoop et al. 2000; Banyal et al. 2015), antipyretic (El-Khatiba and Khaleel 1995; Tiwari et al. 2015; Emon et al. 2021), antitumor (Gupta et al. 2004; Pandey 2017) and antiulcer (Rajkapoor et al. 2003; Del Rey et al. 2018) and analgesic (Filho et al. 1997; Zakaria et al. 2007; Zakaria et al. 2012; Abello et al. 2018) properties have been reported in *Bauhinia spp*.

Bauhinia scandens have a long history of application in traditional medicine. The Ulladan tribal group members of Idukki district, Kerala, India claim the usage of oil prepared from this plant for massaging paralysed children. They also grow this plant in the house premises to keep snakes away, as the Malayalam name indicates, *Nagavalli*, *Naga* means snake *and valli* means vine (Bindu 2016). Despite the promising traditional therapeutic applications of a few Bauhinia species, no scientific studies to support the traditional medicinal applications and phytochemical analysis using sophisticated instruments has not been carried out to the best of our knowledge. Hence present study focuses on the antioxidant and anti-inflammatory activities of an ethanol extract of *Bauhinia scandens* leaves, and gas chromatographic analyses (GC-MS) and UPLC-QTOF-MS of the extract to identify the phytocompounds.

2. Materials and methods

2.1 Sample collection and molecular characterization of plant material

The leaves of *Bauhinia* were collected from Pathanamthitta district, Kerala, India. The leaf samples were transported to the laboratory and stored at − 80 °C.The leaf samples were immersed in liquid nitrogen and crushed using sterile mortar and pestle to get a fine powder.

The template DNA for PCR assay was prepared from the leaf samples by NucleoSpin® Plant II Kit (Macherey-Nagel, Germany). Quality of the extracted DNA was determined using gel electrophoresis. Isolated plant genomic DNA was stored at − 20 °C for further use. PCR was used to amplify the target region of the gene and the amplification was performed with a reaction volume of 25µl containing reaction 10X PCR reaction buffer, 1.5 mM $MgCl_2$, 200 mM of each dNTPs, 1 unit of Taq polymerase (Sigma-Aldrich, Bengaluru, India), 20 ng genomic DNA. An aliquot of 2µl of 10pmol of Forward 5'-ATGTCACCACAAACAGAGACTAAAGC-3' and RBCL-724R Reverse 5'-TCGCATGTACCTGCAGTAGC-3' (Sigma-Aldrich, Bengaluru, India).

The reaction was carried out in a thermal cycler (T100^{TM} Thermal Cycler, Bio-Rad, Singapore), following the protocol detailed by Divya and Karanth (2018). The reaction mixture was initially denatured for 96℃ for 1 minute, subjected to 40 cycles of denaturation at 98℃ for 30s, annealed at 58℃ for 10s, extended at 72℃ for 15s, finally extended at 72 ℃ for 10 minutes and held at 4℃. The amplified PCR products were separated on a 1.5% (w/v) agarose gel by electrophoresis. Respective bands were visualized along with a 100bp DNA ladder. DNA sequence analysis of the representative amplicon was performed at an outsourcing laboratory, 'SciGenom', Kerala, India. The trimmed sequence was assessed using the nBLAST tool in NCBI for their identity.

2.2. Extraction and preliminary phytochemical screening of the extract

The fresh leaves were washed thoroughly. The cleaned plant leaves were dried under shade for 10 days and were ground to coarse powder. The resulting powder was then extracted with ethanol for 24 h with the aid of soxhlet apparatus in 1:10 sample: solvent ratio (Ghasemzadeh et al. 2015; Lozano Grande et al. 2018). The ethanolic extract obtained was filtered and then concentrated and was stored in refrigerator for further studies. The ethanolic leaf extract was subjected to different chemical tests for the detection of phytoconstituents such as alkaloids, carbohydrates, glycosides, sterols, phenolics, tannins flavonoids and proteins, using standard methods (Gupta et al. 2009; Dhale 2011; Pandith 2012; Krishnamoorthy 2015; Singh et al. 2018).

2.3. Quantitative analyses of phenols and flavonoids

Since the phenol and flavonoid contents are the most important among the phytochemical constituents, they were assessed quantitatively. The amount of total phenolics

in the ethanol extract was determined with the Folins –Ciocalteu method (Singleton et al. 1999). Flavonoid determination was carried out by aluminium chloride technique (Humadi and Istudor 2009).

2.4 Gas Chromatography –Mass Spectroscopic (GC-MS) Analysis

The GC – MS analysis was carried out using an Agilent model no:7890 A –Gas Chromatograph coupled to a mass detector of Agilent make -5975c, Gas Chromatograph interfaced to a Mass Spectrometer equipped with a DB 5 ms capillary column (30m×0.25mm × 0.25µm) (Kumar et al. 2019). Injection temperature was maintained at 250^0C and ion source temperature at 230^0C. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 1µLwas employed split ratio of 50:1, the instrument was set to an initial temperature of 40 ˚C and maintained at this temperature for 5 min. At the end of this period the oven temperature was arisen up to 280 ℃ and maintained for 10 min. The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70 eV. The relative % amount of each component was calculated by comparing its average peak area to the total areas (Anjukrishna et al. 2015; Panda et al. 2019) using the software turbo mass.

2.5 Ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS)

Chromatographic separation and determination of analytes were carried out via UPLC-QTOF-MS. The Acquity UPLC system (waters) consists of a TUV detector (J12 TUV 750 A), a column chamber (J12 CHA 730G), a quaternary solvent manager (H12 QSH 632A) and 9 sample manager FTN (K12 SD1069G). A reversed phase BEH C18 column of dimension 50 mm x 2.1 mm x 1.7mm) column with a flow rate of 0.3 mL min^{-1} was used for chromatographic separation (Waters). The mobile phase was a mixture of water and acetonitrile with 0.1%Formic acid in gradient mode. The UHLPC system was connected to the quaruplole time of flight mass spectrometer (Waters Xevo G2 Q TOF) with electrospray ionization. ESI, Interface working in positive and negative ionization modes. The injection volume was 10 µL. All samples were analysed using ESI negative ionization mode, scanning the m/z range between 50 and 1000.The desolvation gas flow and the temperature were 900 L/h and 350 °C respectively. The mass spectra were obtained using collision energy ranging from 5 to 30 eV. The instrument control and data acquisition was done using MassLynx Software (v 4.1).

2.6. In vitro Antioxidant Activity

The antioxidant activity of the leaf extract was evaluated using phosphomolybdenum method and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity.

Total Antioxidant Activity

Total antioxidant activity of the extract was determined according to the method of Prieto et al. 1999. 0.3 mL of each fraction was mixed with 3.0 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Reaction mixture was incubated at 95^0C for 90 min in a water bath. Absorbance of all the sample mixtures was measured at 695 nm. Ascorbic acid (100 µg/mL) was used as standard control.

Free Radical Scavenging Activity by DPPH

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was evaluated according to Negi et al. 2003 with little modification. 0.1 mL of the extract was treated with methanolic solution of DPPH (1.4 mL; 0.2mM) and 1.5 mL of distilled water, mixed thoroughly in vortex and the mixture was placed in the dark for 30 min. The decrease in absorbance was measured at 515 nm against a blank using a spectrophotometer. Control tube containing he same reagents without plant extracts which was incubated under the identical conditions. Percent DPPH inhibition was calculated as Lee et al. 2002 as follows:

% of inhibition = (Absorbance of control – Absorbance of sample)/ Absorbance of control) x100

2.7. Anti-inflammatory activity

The anti-inflammatory activities of the extracts were determined as described by Williams et al. (2008) using BSA assay with slight modification. BSA solution (0.4% w/v) was prepared in Tris buffered saline. The pH was adjusted to 6.4 with glacial acetic acid. Stock solutions of plant extract were prepared in ethanol at a varying concentration. Respective aliquots from stock solutions were added to test tubes containing 1 mL of 0.4%, w/v BSA buffer solution. Both negative (ethanol) and positive (aspirin) controls were assayed in a similar manner. The solutions were then heated in a water bath at 72 °C for 10 minutes, and cooled for 20 minutes under laboratory conditions. The turbidity of the solutions (level of protein precipitation) was measured at 660 nm. The percentage inhibition of precipitation (protein denaturation) was determined using the following equation:

% inhibition= 100*(Absorbance of Control-Absorbance of test/Absorbance of control)

3. Results

3.1. Sample information and molecular identification

Bauhinia scadens leaves (Fig.1) collected were morphologically identified and authenticated by Dr. A. K. Pradeep, Assistant Professor, University of Calicut. Molecular identification confirmed that the plant was *Bauhinia scandens* and the gene sequences were submitted to the GenBank and accession number is *BankIt2464393 Seq1 MZ269001*. Molecular identification using rbcl gene sequencing also in agreement with the identification by morphological characteristics.

3.2. Extraction and Phytochemical screening

The ethanolic extraction yield of the leaf sample using soxhlet method was found to be 12.17±0.96 %. The presence of phytocompounds such as alkaloids, carbohydrates, phenols, flavonoids, steroids and glycosides were reveled on the qualitative analysis of phytocompounds. Table 1 represents the phytochemical compounds present in the ethanolic extract of *B. scandens* (Table 1).

3.3. Quantitative analysis of phenolics and flavonoids

B scandens leaf extract has high phenolic contents (23.381± 0.0930 µgGAE/g of extract) and flavonoid contents (9.087227± 0.0440 µgQE/g of extract). TPC was calculated using the standard curve of gallic acid (standard curve equation: $y = 0.0916x - 0.1627$ with R^2 : 0.9904) and TFC was calculated using the standard curve of quercetin (standard curve equation: $y = 0.0107x + 0.0121$ with $R^2 = 0.9946$). The corresponding results are listed in table 2.

3.4. *In vitro* Antioxidant activity

Total antioxidant activity of the extract was analyzed by the phosphomolybdate method and the result was expressed in ascorbic acid equivalents. The EC_{50} value for the total antioxidant capacity for the ethanol extract (23.93±0.12 µg/mL) was comparable with that of the standard (ascorbic acid: 24.73±0.09 µg/mL) (Table 3). Fig. 2 illustrate the total antioxidant activity of the ethanolic extract of *B. scandens*

The IC_{50} values of scavenging DPPH radicals for the extract and standard (ascorbic acid) were 39. 64 ±0.02 µg/mL and 37.40 ±0.50 µg/mL, respectively. Antioxidant activity of the extract could be attributable to the presence of phenolic compounds. Fig. 3. shows the scavenging effects of the extract on DPPH radical.

3.5. *In vitro* anti-inflammatory activity

In vitro anti-inflammatory activity of the plant extract was analysed. The results were expressed as IC_{50} (Inhibition concentration at 50% inhibitory activity). Aspirin was used as standard in comparison with the extracts. Fig.4. shows the responsive curve of the extract. The IC_{50} values for the extract and standard were 32.22±0.50 µg/mL and 36 ±0.20 µg/mL, respectively. The lower the IC_{50} value, the higher the anti-inflammatory activity of sample.

3.6. GC-MS analysis of *B. scandens* extract.

GC-MS analysis of the ethanol extract revealed the presence of seven major compounds with specific retention time (RT), molecula formula (MF), molecular weight (MW) and concentration (% peak area) as shown in Table 3. In terms of % peak area, n gamma-Cyano-3-methyl-5,10-dihydrobenzo[f] indolizine (17.29), Cinnamamide, N,.alpha.-diphenyl-beta-methyl-(8.19), 3-Hydroxy-7,8-dihydro-.beta.-ionol (7.29), Benzoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester (22.09), Urocanic acid, N,O-bis(trimethylsiyl)-(10.41) were found to be the major compounds, whereas [1,2,4]Triazolo[1,5-a]pyrimidine-carboxylic acid, 7-amino-, ethyl ester (12.00) and Hexahydropyridine, 1- (7.95) are the major compounds. From the study, Benzoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester indicated the highest peak value in the extract with RT: 41.280 mins and % peak area: 22.09 %.

3.7. UPLC-QTOF-MS Characterization

Sixteen compounds were identified in UPLC-QTOF-MS spectral analysis and their properties and biological activities are listed in table 4. The compounds obtained were 3-O-Methyl gallate, Syringate, 4-Acetyloxy-3,5-dimethoxybenzoate, Palmitate/Hexadeconate, Kaempferol derivative, Quercetin-7-olate, Quercetin-3-olate, Genistein, 5,7,4'-triOH-3,6,8,3'-tetraOMe flavones, Isovitexin, Vitexin, Quercetin-3-glucoside (Isoquercetin), Vitexin 2"-O-beta-D-glucoside (1-), Rutin, Quercetin-(galloyl)glucoside and Gallocatechin / Epigallocatechin.

4. Discussion

The analysis of the chemical composition of the genus *Bauhinia* sp. demonstrated the presence of bioactive phytocompounds such as terpenes, steroids, alkaloids and particularly flavonoids (Silva and Cechinel Filho 2002). *B. scandens* is the least explored species in this genera. Pizzolatti et al. 2003 isolated various flavanoids from the leaf extract of *B. forficata*, including kaempferol and other flavonoid glycosides. Pinheiro et al. 2006 developed a chromatographic method to quantitatively determine phytocompounds in medicinal extracts of *Bauhinia sp* leaves (Achenbach et al. 1988). The current study also throw insight in to the anti-inflammatory and anti-oxidant potential of ethanolic leaf extract of *B. scandens* and it might be due to the presence of important phytocompounds. GC-MS and UPLC-QTOF-MS characterization finally supported the prevalence of important biomolecules like vitexins, isoflavones, quercetins, rutins, syringate, gallocatechins, kaempferols, alkaloids, glucosides etc. in the ethanolic extract of *B. scandens*.

DPPH radical scavenging is a widely used method to evaluate the free radical scavenging activity of compounds or antioxidant capacity of plant extracts. Antioxidants are molecules that may scavenge free radicals, suppress lipid peroxidation, chelate metal ions and acquire reducing power, thereby protecting human body from oxidative stress (Arora and Chandra 2011; Rashed and Butnariu 2014). A large variety of antioxidants have been proposed to be used in the management of various human diseases (S. Cuzzocrea et al. 2001). Plant originated antioxidants showed advantageous as they are less cytotoxic and more effective cum economical, and therefore there is growing demand in natural antioxidants (Sowndhararajan and Kang 2013; Shahana and Nikalje 2017). Total antioxidant activity by phosphomolybdenum method revealed an EC50 value of 14.33±0.12 µg/mL for ethanol extract of *B. scandens* which was less than EC50 value of the standard (24.73±0.09 µg/mL) and hence we can conclude that the total antioxidant activity of ethanolic extract of *B. scandens* is comparable to ascorbic acid. The result was also validated by DPPH assay and showed that an IC_{50} value of 39.8 ±1.00 µg/mL for the extract and 37.40 ±0.50 µg/mL for standard. It was already reported that the presence of phenol and flavonoids contribute to antioxidant activity of the extract (Hossain t al. 2016). Mishra et al. 2013 reported that the antioxidant activity of the leaf extracts of *Bauhinia variegata* was concentration (dose) dependant. Polar extracts exhibited appreciable metal ion chelating activity at lower concentrations. Aqueous and methanolic extracts exhibited strong to moderate free radical scavenging activity. This data scientifically support the basis

for their exploitation in traditional medicine for healing wounds and also in treatment of some communicable diseases (Avinash et al. 2011; Cagliari et al. 2018; Santos et al. 2019). Hossain et al. 2016 also reported the antioxidant potency of *Bauhinia scandens* and found an IC50= 13.5 µg/ml for ethanolic extract.

Anti-inflammatory activity in *Bauhinia* was well documented. In vitro anti-inflammatory activity was expressed as IC50 and aspirin was used as standard and observed that the inhibitory activity was less and 36±0.20 µg/mL was required for the extract for inhibition while 32.22±0.50 µg/mL was only needed for standard. Yadava and Reddy (2003) discovered a novel flavonol glycoside, with anti-inflammatory potential in *B. variegata*. From aerial section of the same plant, Rao et al. (2008) also isolated several flavonoids along with other phytocompounds which act as inhibitors of various macrophage functions involved in the inflammatory process. Racemosol and 10-O-demethylracemosol, which are biologically active and can function as efficient anti-inflammatory molecules were also reported in *B. malabarica* (Songarsa et al. 2005). Boonphong et al. (2007) isolated numerous new and unidentified metabolites, some with anti-inflammatory activity from the roots of *B. purpurea* plant. Several other reports have been published in concern with anti-inflammatory activity of Bauhinia plants (Das et al. 2012; Al-Taweel et al. 2015; Campos et al. 2016). In the light of literature sources and our study, it is concluded that ethanol extract of *Bauhinia scandens* leaves could be a potential antioxidant as well as anti-inflammatory agents, which can be further utilized for therapeutic purposes.

GC and UPLC-QTOF-MS characterization of *B. scandens* extract revealed the presence of various bioactive compound and detected phytocoponents have potential activities which were already well documented except for Urocanic acid, N,O-bis(trimethylsiLyl, which were reported for the first time in plants.

5. Conclusion

This study give insight in to the medicinal properties of *Bauhinia scandens* and proved efficacy of ethanolic extract of *Bauhinia scandens* as an excellent source of antioxidants and showed anti-inflammatory activity. GC and UPLC-QTOF-MS characterization of *B. scandens* extract revealed the presence of various phytocompounds of biological importance.

Table 1. Qualitative phytochemical screening for ethanolic extract of *B. scandens.*

Test	**Presence or absence**
Wagners's/ Mayer's test for alkaloids	+
Molisch 's test for carbohydrates	+
Keller killiani test for glycosides	+
Salkowski tests for sterols	+
Ferric chloride test for phenols	+
Gelatin test for tannins	-
Alkaline reagent test for flavonoids	+
Biuret test for proteins	-

Note: Symbol + and - indicate Presence and absence of phytocompounds respectively.

Table 2. Quantitative analysis of phenols and flavonoids *B. scandens* extract.

Sl no	**Phytochemicals**	**Results in standard equivalents/g**
1	Total phenol	23.381± 0.0930 µg GAE/g
2	Total flavonoid	9.087227± 0.0440 µgQE/g

Note: GAE- Gallic acid equivalents and QE- Quercetin Equivalents respectively.

Table 3. Total antioxidant activity of ethanolic extract of *B. scandens*

Sl no	Sample (in 50 µg/mL)	OD at 695 nm	Total antioxidant activity expressed in terms of EC_{50} Value
1	Standard (ascorbic acid)	0.607	24.73±0.09 µg/mL
2	Ethanol extract of *B. scandens*	0.986	23.93±0.12 µg/mL

Table 4. GC-MS profile of phytocomponents in *B. scandens.*

Sl No:	Retention Time	Phytocompound	Peak %	Biological activity
1	36.831	gamma-Cyano-3-methyl-5,10-dihydrobenzo[f] indolizine	17.92	Anti-inflammatory, analgesic, antimicrobial, antiexudative, anti inflammatory, anti-tumour (alkylating) agents and hypoglycemia activities (Vemula *et al*., 2011; Venugopala *et al*., 2017)
2	39.457	Cinnamamide, N,.alpha.-diphenyl-beta-methyl- (Cinnamic acid is an organic acid occurring naturally in plants)	8.19	Well-known antioxidants, Antimicrobial activity, immunomodulatory, antimicrobial, antiviral, larvicidal, insecticidal, diuretic, pungent, analgesic and cannabimimetic (Sova *et al*., 2012; Madhavi *et al*., 2019)
3	39.779	3-Hydroxy-7,8-dihydro-.beta.-ionol (Disaccharide glycoside)	7.29	Aroma precursor (Ma *et al*., 2001; Aubert *et al*., 2003; Selli *et al*., 2004)

4	41.280	Benzoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester	22.09	Antibacterial, antioxidant, anticancer, antiseptic, antiviral and hepatoprotective (Vimalavady *et al*., 2013)
5	43.494	Urocanic acid, N,O-bis(trimethylsi lyl)- (Presence in plants is not reported)	10.41	Photoprotectant
6	45.480	[1,2,4]Triazolo[1,5-a]pyrimidine-carboxylic acid, 7-amino-, ethyl ester (pyrimidine derivative)	12.00	Antitumor and antileukemia activity (Abunada *et al*., 2008)

Table 5. UPLC-QTOF-MS characterization of *B. scandens* extract.

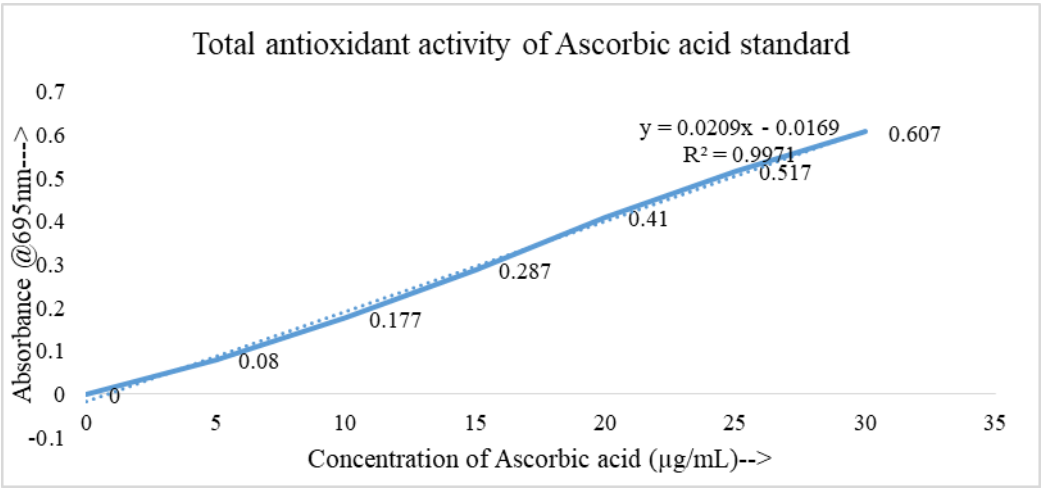
Sl no	**RT**	**Experimental Parent Ion (Ionization mode)**	**Compound**	**Molecular formula**	**Biological activity**	**References**
1	2.56	183.0291 (M^-)	3-O-Methyl gallate	$C_8H_7O_5$	Antioxidant, antiapoptotic and anti-inflammatory properties	Rosas et al. 2019
2	3.32	197.0449 (M^-)	Syringate	$C_9H_9O_5$	Antioxidant, anti-inflammatory, and	Kahkeshani et al. 2019

					antineoplastic properties	
3	2.96	239.0564 (M^-)	4-Acetyloxy-3,5-dimethoxybenzoate	$C_{11}H_{11}O_6$	Antibacterial and Anticancer activities	Xiong et al. 2021
4	3.65	252.2324 (M^+)	Palmitate/ Hexadeconate	$C_{16}H_{31}O_2$	Neuroprotective, anti-neuro inflammatory and analgesic activities	Carta et al. 2017
5	3.46	287.0552 (M^+)	Kaempferol derivative	$C_{15}H_{10}O_6$	A natural flavonoid compound with anticancer, antioxidant and antiapoptic activity	Chen and Chen 2013
6	3.23	300.9982 (M^-)	Quercetin-7-olate	$C_{15}H_9O_7$	Antioxidant, Anti-inflammatory, Anticancer and Antihistamine effects	Li et al. 2016
7	3.15	303.0506 (M^+)	Quercetin-3-olate	$C_{15}H_9O_7$	Antioxidant, Anti-inflammatory and Anticancer effects	https://www.healthline.com/ nutrition/quercetin
8	3.665	349.0558 (M^-)	Genistein	$C_{15}H_{10}O_5$	An isoflavanone with anti-helminthic, anti-cancer, anti-diabetic and anti-	Tandon and Das 2018

					oxidant properties	
9	3.30	391.0667 (M^-)	5,7,4'-triOH-3,6,8,3'-tetraOMe flavones	$C_{20}H_{20}O_8$	Antioxidants, anticancer, antibacterial, cardioprotective agents and anti-inflammatory properties	Tungmunnithum et al. 2018
10	3.46	431.0974 (M^-)	Isovitexin (also called as homovitexin or saponaretin)	$C_{21}H_{19}O_{10}$	An apigenin-6-C-glucoside flavonoid compound with antioxidant and helps in skin protection preventing from damage by enhancing the stem cell properties of keratinocytes	Chowjarean et al. 2019
11	3.26	433.0751 (M^-)	Vitexin	$C_{21}H_{20}O_{10}$	An apigenin-6-C-glucoside flavonoid compound with anti-oxidant, anti-cancer, anti-inflammatory, anti-hyperalgesic, and neuroprotective effects	He et al. 2016
12	3.15	463.0885 (M^-)	Quercetin-3-glucoside (Isoquercetin)	$C_{21}H_{19}O_{12}$	Antibacterial, antithrombotic, vasodilatory, anti-inflammatory, and	Maldonado-Celis et al. 2019

					anticarcinogenic effects	
13	3.20	593.1492 (M^-)	Vitexin 2"-O-beta-D-glucoside (1-)	$C_{27}H_{29}O_{15}$	Antioxidant, neuroprotective and hepatotoprotective activities	Abdulai et al. 2020
14	3.19	609.1461 (M^-)	Rutin	$C_{27}H_{29}O_{16}$	Powerful antioxidant and anti-inflammatory effects	Ganeshpurkar and Saluja 2017
15	3.17	615.0983 (M^-)	Quercetin-(galloyl)glucoside	$C_{28}H_{23}O_{16}$	Antihistamine, anti-inflammatory, antidiabetic and antioxidant activities	Matsuzaki 2010 and Park et al. 2017
16	3.18	761.1538 (M^-)	Gallocatechin / Epigallocatechin	$C_{37}H_{29}O_{18}$	Chemical secondary metabolite reported from *Bauhinia sp* having anticancer property against many types of cancer	Aquino et al. 2019

Figure1. Leaf sample of *Bauhinia scandens*



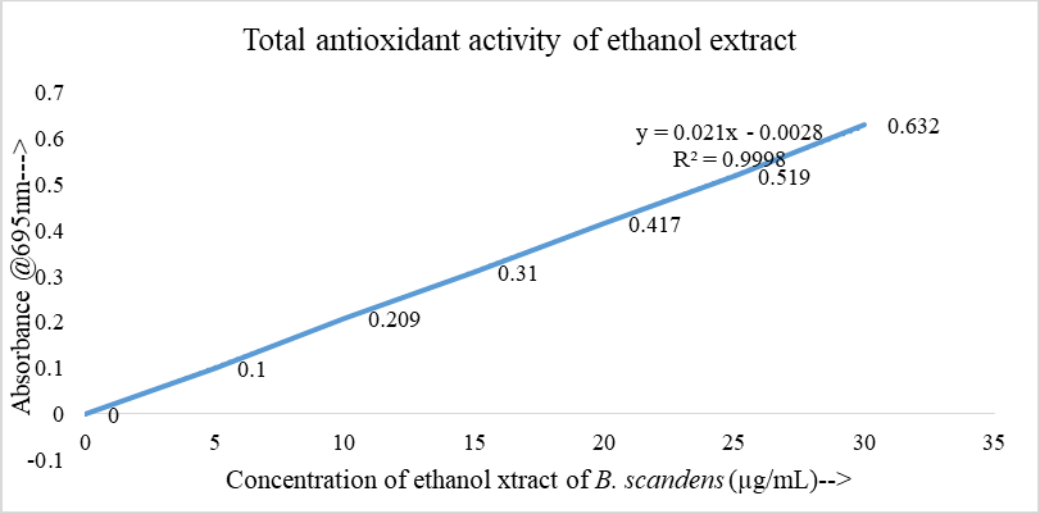
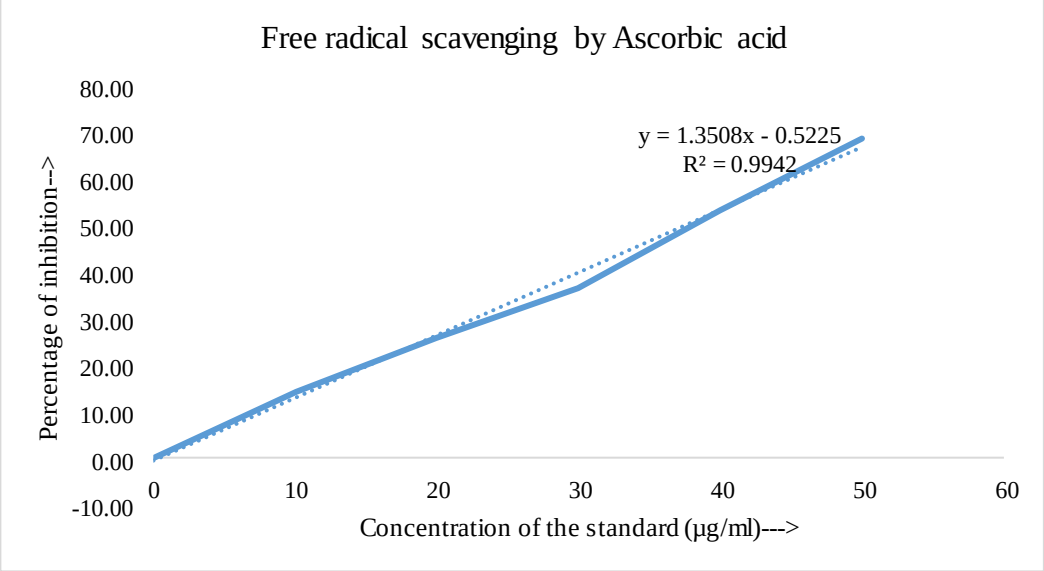


Figure 2. Total antioxidant activity of Ascorbic acid (Reference standard) and ethanol extract of *B. scandens*



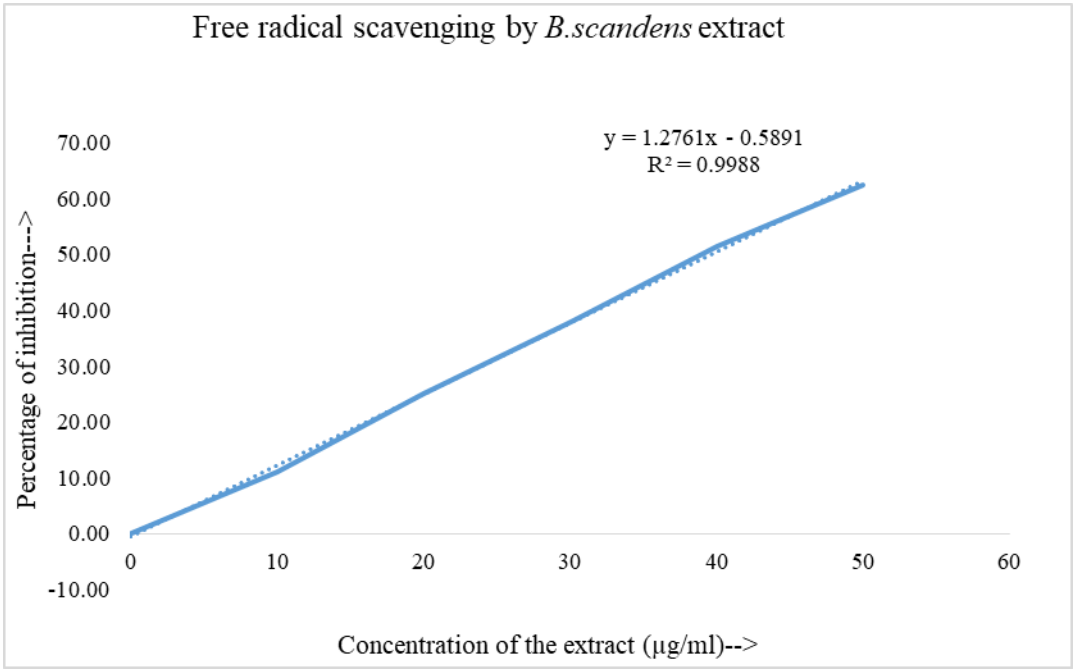


Figure3. Dose responsive curve of DPPH free radical scavenging activity of Ascorbic acid (Reference standard) and ethanol extract of *B. scandens.*

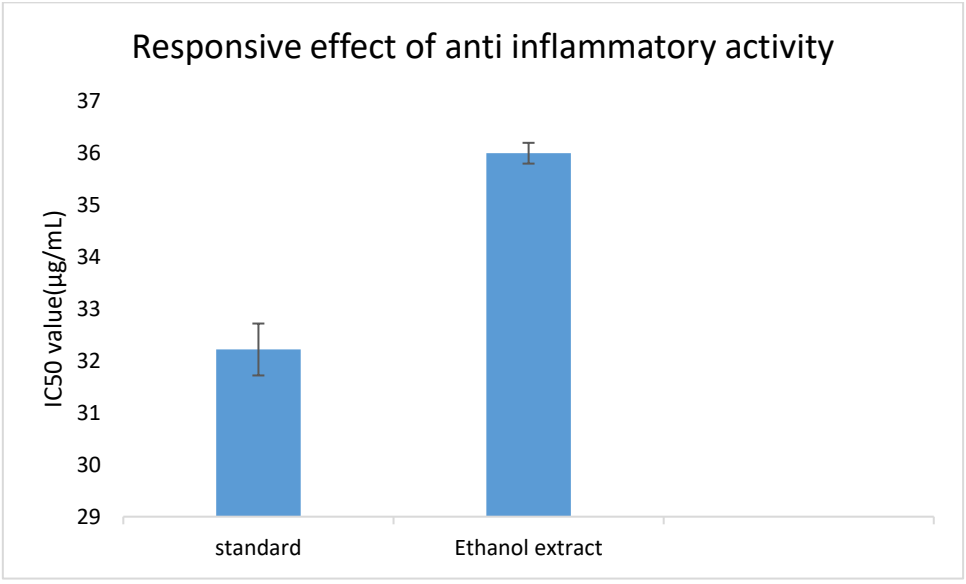


Figure 4. Dose response curve of anti-inflammatory activity *B.scandens* extract

6. Conflict of Interest

The authors whose names are listed certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non financial interest in the subject matter or materials discussed in this manuscript.

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