Research Article

Phytochemical and Pharmacological Investigation on *Onosma bracteatum* Wall.

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ABSTRACT

Introduction: *Onosma bracteatum* is one of the important species of *genus Onosma* growing in Himalayas in India that has been used traditionally as a beneficial remedy for many diseases yet in unexplored scientifically. **Objectives:** The aim of study is to isolate and quantify compound and evaluation of antioxidant and cytotoxic potential of extract and fractions of whole plant of *O. bracteatum*. **Materials and Methods:** Total methanolic extract (TME) was prepared by Sohxlet extraction method and Successive solvent extraction of plant material was performed using Soxhlet apparatus to yield petroleum ether (A1), chloroform (A2), ethyl acetate (A3), n-butanol (A4) and water (A5) extracts. TME was subjected to qualitative phytochemical screening and determination of total phenolic content (TPC) and total flavonoid content (TFC). A1 was subjected for column chromatography and isolated compound was characterized by spectral analysis and quantified using HPTLC. Total antioxidant capacity (TAC), free radical scavenging activity (DPPH), nitric oxide scavenging assay and cytotoxicity through Brine Shrimp Lethality Assay (BSLA) of TME and fractions of *O. bracteatum* were conducted. **Result and Discussion:** Results of the study showed the presence of quinones, alkaloids, flavonoids, phenolics, coumarins, fixed oil and fat, carbohydrates and terpenoids in the TME of *O. bracteatum*. The TPC and TFC of *O. bracteatum* were found 14.5±0.00047 mg GAE/g of dry powder and 11.2±0.0014 mg RUE/g of dry powder respectively. Probable structure of isolated compound was 1,7-dihydroxy-3-methyl-9H-xanthene- 9-one and the amount of compound was found to be 0.17 ± 0.11 %w/w in the plant. Total antioxidant capacity was found higher in A4 (58.56±0.0015 µg BHTE/mg of dry extract). TME showed lowest EC_{50} value for the DPPH radical scavenging activity TME showed significantly higher activity compared to the A1, A2, A3, A4 and A5. The fraction A4 showed significantly higher nitric oxide scavenging activity compared to the TME, A1, A2, A3 and A5. For cytotoxicity test, A4 (IC_{50} =74.13µg/ml), A1 (IC_{50} =75.85µg/ml), A2 (IC_{50} =79.43µg/ml) showed equivalent cytotoxicity compared to TME, A3 and A5. The results indicate that the A4 may contain higher phenolics and flavonoids, on other hand A1 and A2 are rich in alkannin/shikonin and xanthon type of compounds in *O. bracteatum*. **Conclusion:** *O. bracteatum* possessed significant antioxidant and cytotoxic potential.

Key words: BSLA, DPPH, HPTLC, *Onosma bracteatum*, Phytochemicals.

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INTRODUCTION

Medicinal plants are in sought after as a primary source for lead molecules in drug discovery. According to one report statistically defined chemical space is similar for natural products and drugs, while combinatorial compounds and drugs do not share similar space.[1] The recent advancement in science has opened up new avenues and facilitated search of the new therapeutic agents from medicinal plants that are used in traditional medical practices.[2,3] Plant selection is corner stone in drug discovery and the systematic approach of exploring traditional, ethno-medicinal, ethno-pharmacological or ethno-botanical literature is usually opted for.[4] The present study is one such effort and investigates a traditionally valued indigenous medicinal plant *Onosma*

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bracteatum Wall. (Family: Boraginaceae) commonly known as 'Gaozaban'/ 'Gojihva'). The plant is valued as a tonic in building the body immune resistance, spasmolytic and diuretic and is indicated in the treatment of asthma, bronchitis, rheumatism, irritation of bladder, syphilis, leprosy, wound and skin diseases.[5-8] Documented records on *O. bracteatum* is suggestive of its antioxidant,[9] anti-inflammatory and antiallergic,[10] antidiarrhoeal,[11] psycho-immunomodulatory,[12] wound healing,[13] antibacterial,[14] analgesic,[15] anticancer[16] and anti-ageing[17] activities. In the event of inconsequential literature on both pharmacological and phytochemical aspects we here propose bioactivity guided extraction of *O. bracteatum* with specific reference to evaluation of its antioxidant and cyototoxic potential.

MATERIALS AND METHODS

Sample collection and preparation

The plant material was collected and authenticated by Dr. V. P. Bhatt (Taxonimist) from Vyans valley, Pithoragarh, Uttarakhand and herbarium specimen number: LMCP/PCG/223 is deposited at the Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad, Gujarat, India. The collected plant material was freed of soil and dust, dried, powdered and stored in an airtight glass container until required for extraction.

Preparation of Extracts

100g of dried powdered plant material was extracted with the 400ml of methanol using continuous Sohxlet extraction method to yield methanolic extract (TME) as sticky brown semisolid. 100g of dried powdered material was subjected to successive solvent extraction using petroleum ether, chloroform, ethyl acetate, n-butanol and water. After evaporation of solvents under vacuum the dried extracts obtained were labeled as pet. Ether (A1), chloroform (A2), ethyl acetate (A3), n-butanol (A4) and Water (A5) and were kept in refrigerator till required for further analyses.

Phytochemical Analysis

Qualitative Phytochemical Screening

TME was screened for presence of phytochemicals visually, alkaloids (pyrrolizidine),[18] quinones, phytosterols, phenolics, saponins, flavanoids, coumarins, proteins and carbohydrates.[19]

Quantitative Phytochemical Screening

Total Phenolic Content (TPC)

The assay was determined using Folin-Ciocalteu Method using gallic acid as a reference standard.[20] The total phenolic content was calculated from the calibration curve of gallic acid and the results were expressed in mg gallic acid equivalent per gram of dried extract. The estimation was performed in three trials and the results were expressed in mean ± SD.

Total Flavonoid content (TFC)

The flavonoids content was determined using aluminum chloride colorimetric method using rutin as a reference standard.[21] Absorbance was measured at 415 nm against the suitable blank. From a calibration curve of rutin, the total flavonoid content was calculated and the result was expressed in mg rutin equivalent per gram dry weight extract. The determination was performed in three trials and the results were expressed in mean ± SD.

Isolation of compounds from A1

A1 (0.250g) was subjected to silica gel column chromatography for the isolation of phytoconstituents. The column was packed with silica gel (60-120#) as the stationary phase, the extract was loaded on the column and gradient elution was performed using *n*-hexane: ethyl acetate. A total of 105 fractions, each of 10ml were collected and simultaneously studied on TLC. Fractions with similar pattern on TLC were pooled, concentrated and then allowed to evaporate to dryness. The fractions 29-40 (2.0% EtoAc in *n*-hexane) that were pooled, on evaporation yielded an orange solid (coded as compound I) when subjected to TLC using *n*-hexane: ethyl acetate: methanol (9.8:0.2:0.1) mobile phase showed single spot, the isolated compound was subjected for the spectral analysis like UV, IR, MASS and NMR for deriving structure.

HPTLC method development for quantification of Compound I

Preparation of standard stock Solution of Compound-I

Compound-I (2mg) was weighed accurately and transferred into 5mL vial and dissolved using dichloromethane and make up volume up to 4mL (S1=0.5mg/mLof Compound-I)

Preparation of Test solution

20mg of A1 (pet. ether) extract was dissolved in 1.5mL dichloromethane (T1=13.33mg/mL)

Chromatography

HPTLC was performed on 10cm × 10cm precoated silica gel 60 F_{254} plates (E. Merck, Darmstadt, Germany). The plates were prewashed by methanol and activated at 60°C for 5min. The samples were applied to the plates as bands 6mm wide and 10mm apart using a CAMAG Linomat V applicator (CAMAG, Muttenz, Switzerland)

fitted with a 100µL syringe (CAMAG, Muttenz, Switzerland). The plate was developed using *n*-hexane: ethyl acetate: methanol (9.8:0.2:0.1v/v/v) as the mobile phase. After drying, the plates were derivatized using 10% methanolic KOH followed by heating at 105°C for 10min and scanned in a CAMAG TLC Scanner using win- CATS software at 254nm with slit dimensions 4 × 0.1mm. The scanning speed was 20mm s^{-1}, and the source of radiation was a tungsten lamp.

Calibration curve of Compound-I

Graded concentration of standard solutions (0.5mg/mL) in 0.6, 1.0, 1.4, 1.8 and 2.2 µL volume were applied on plate. The concentration of compound-I was 300, 500, 700, 900, 1100ng/band. The plate was developed in mobile phase *n*-hexane: ethyl acetate: methanol (9.8:0.2:0.1v/v/v) and scanned at 254nm. Data of peak area of each Compound-I was recorded. The calibration curve was obtained by plotting curve area vs. concentration of each peak corresponding to the respective band.

Estimation of Compound-I in sample of *O. bracteatum*

2µL of test solution was spotted along with working standard solution of 0.6, 1.0, 1.4, 1.8 and 2.2µL on plate. The plate was developed in mobile phase *n*-hexane: ethyl acetate: methanol (9.8:0.2:0.1v/v/v) and scanned at 254nm. The peak areas were noted, and quantification was performed using linear regression equations.

In vitro antioxidant assays

Total Antioxidant Capacity

The total antioxidant capacity of the extracts was evaluated according to the method described by Prieto *et al*.[22,23] and was expressed as µg equivalents of BHT by using the standard BHT graph. The estimation was performed in three trials and the results were expressed in mean ± SD.

DPPH Free radical Scavenging Assay

The DPPH scavenging potential of different extracts of *O. bracteatum* was measured based on scavenging ability of stable 1,1 diphenyl-2-picrylhydrazyl (DPPH) radicals. Freshly prepared 2ml DPPH solution (33mg/L) was mixed with 2ml of different solution of *O. bracteatum* extracts. The reaction mixture was stand for 15min in at room temperature in dark place. Absorbance of resultant mixture was recorded at 517nm using UV visible spectrophotometer.[24] The percentage of DPPH scavenging by the extract and standard compounds was calculated by using following formula:

$$\% \text{ Inhibition} = \frac{(A_{control} - A_{test})}{A_{control}} \times 100$$

Where, $A_{control}$ is Absorbance of Control, A_{test} is Absorbance in presence of the sample of extract and standard. Plotted the graph percentage inhibition Vs concentration and calculated the $IC_{50.}$ The estimation was performed in three trials and the results were expressed in mean ± SD.

Nitric Oxide Scavenging Assay

Scavengers of nitric oxide compete with oxygen leading to reduce production of nitrite ions. In aqueous medium, sodium nitroprussside (in PBS) generates NO and nitrite ions on reacting with oxygen subsequently, which is estimated using Griess reagent. The reaction mixture contained 0.3ml sodium nitroprusside (10mM) in phosphate buffer saline and extract at different concentrations (100-700µg) incubated at room temperature for 150min. The same reaction mixture without the tested samples but with equivalent amount of solvent served as control. After the incubation 1ml Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthy lenediamine dihydrochloride) was added. The absorbance of reaction mixture was measured at 546nm.[25] The percentage of Nitric oxide scavenging by the extract and standard compounds was calculated by using formula as mention in DPPH free radical assay. The estimation was performed in three trials and the results were expressed in mean ± SD.

Cytotoxic Activity

Brine Shrimp Lethality Assay (BSLA)[26]

BSLA was employed in the determination of possible cytotoxic activity of *O. bracteatum* Extracts. Briefly the cysts of *Artemiasalina* were hatched in an illuminated, aerator aided separating funnel containing 0.33% sea salt medium (Natural Sea salt mix, Oceanic Systems, Texas, U.S.A.) with some minor modifications. After incubation of 24h, 10 nauplii were harvested and use for the assay. The extracts were tested in triplicates up to a total volume of 5ml, 10 nauplii were added. Extracts were administered at 1000, 500,100 10 and 1µg/mL. Negative and positive controls were run using seawater with DMSO and potassium dichromate, respectively. The number of surviving shrimps were counted and recorded after 24h. The percentage mortality of nauplii was then calculated. There were 3 replicates for each concentration of test solution.

% Mortality = [Number of dead nauplii/ Total number of nauplii] x 100

Using the Probit Table of Finney,[27] the corrected percentage mortality values were transformed to probit values. LC_{50} values were estimated using a probit regression analysis.[28]

RESULTS

Percentage yield of Successive solvent extraction

% yield of extract and fractions of *O. bracteatum* are presented in Table 1.

Qualitative Phytochemical Screening

The Results of the phytochemical screening of TME of *O. bracteatum* are presented in Table 2.

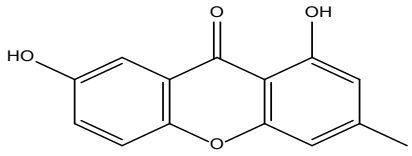
Quantitative Phytochemical Screening

The TPC and TFC in the TME of *O. bracteatum* are presented in Table 3.

Structure elucidation of Compound -I

Compound -I was isolated from column chromatography of A1 as orange solid (15mg). UV spectra with the absorption at 254nm and 430nm indicated the presence of aromatic ring and these are also characteristic peak for quinones (Figure 1). The IR spectra showed the characteristic absorption band at 1227cm^{-1} due to ether linkage, aromatic C-H stretching band observed between 3066cm^{-1} in IR region, –C=O stretching absorption band observed in between of 1688cm^{-1}, broad absorption band obtained at 3421cm^{-1} confirms the phenolic -OH present (Figure 2). The molecular formula $C_{14}H_{10}O_4$ was established on basis of the mass spectrum showing the molecular ion peak m/z (relative intensity) - 242g/mol and base peak at m/z 199g/mol (Figure 3). Protons of $–CH_3$ group were detected at 2.47ppm, All aromatic protons (Ar-H) signal observed between the ranges of 7.11-7.86ppm, Moreover, phenolic hydroxyl groups present in proposed compound was conformed on the bases of peaks seen at δ=12.02 and 12.13ppm (Figure 4).

Probable structure and molecular formula of compound-I



1,7- dihydroxy-3-methyl-9H-xanthene-9-one

Molecular weight: 242g/mol

Molecular formula: $C_{14}H_{10}O_4$

Quantification of Compound –I

HPTLC studies revealed purity of the compound-I resolving at R_f 0.64 and content of compound-I was found to be 0.17 ± 0.11%w/w in the plant (Figure 5).

Total anti-oxidant capacity

Total antioxidant activityof TME and fractions of *O. bracteatum* are presented in Table 4.

DPPH Free radical and Nitric oxide scavenging assay

EC_{50} value (µg/ml) for the DPPH radical scavenging and NO scavenging activity of extract and fractions of *O. bracteatum* are presented in Figure 6.

Brine Shrimp Lethality Assay (BSLA)

The cytotoxic potential of the extracts and fractions of *O. bracteatum* expressed as median lethal concentration (LC_{50}) is shown in Table 5.

DISCUSSION

Alkaloids, quinones, flavanoids, phenolics, phytosterol, fixed oil and fat, coumarins and carbohydrates were detected in TME of *O. bracteatum* (Table 1). Total

Table 1: % Yield of Fractions.

Extract	Colour	% Yield
TME	Reddish brown	18.3
Petroleum ether	Orange Red	1.4
Chloroform	Reddish Brown	2.2
Ethyl acetate	Brownish Yellow	0.82
Butanol	Brownish Black	8.6
Water	Dark brown	12.2

Table 2: The Results of the phytochemical screening of TME of *O. bracteatum*

Test	Total Methanol Extract
Alkaloids	+
Quinones	+
Glycosides	-
Phytosterols	+
Fixed oil and Fats	+
Saponins	-
Phenolic compounds	+
Flavonoids	+
Coumarins	+
Protein	-
Carbohydrates	+

+: Present, - : Absent

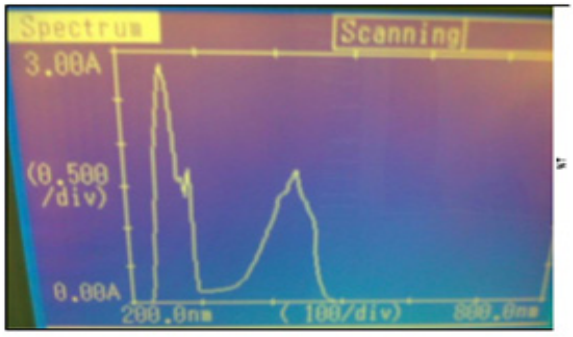


Figure 1: UV spectra of Compound-I.

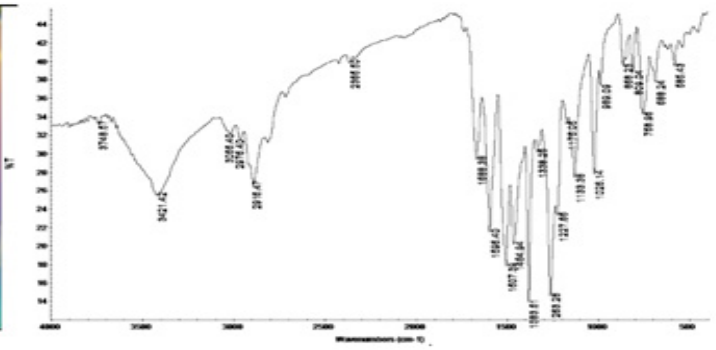


Figure 2: IR spectra of Compound-I.

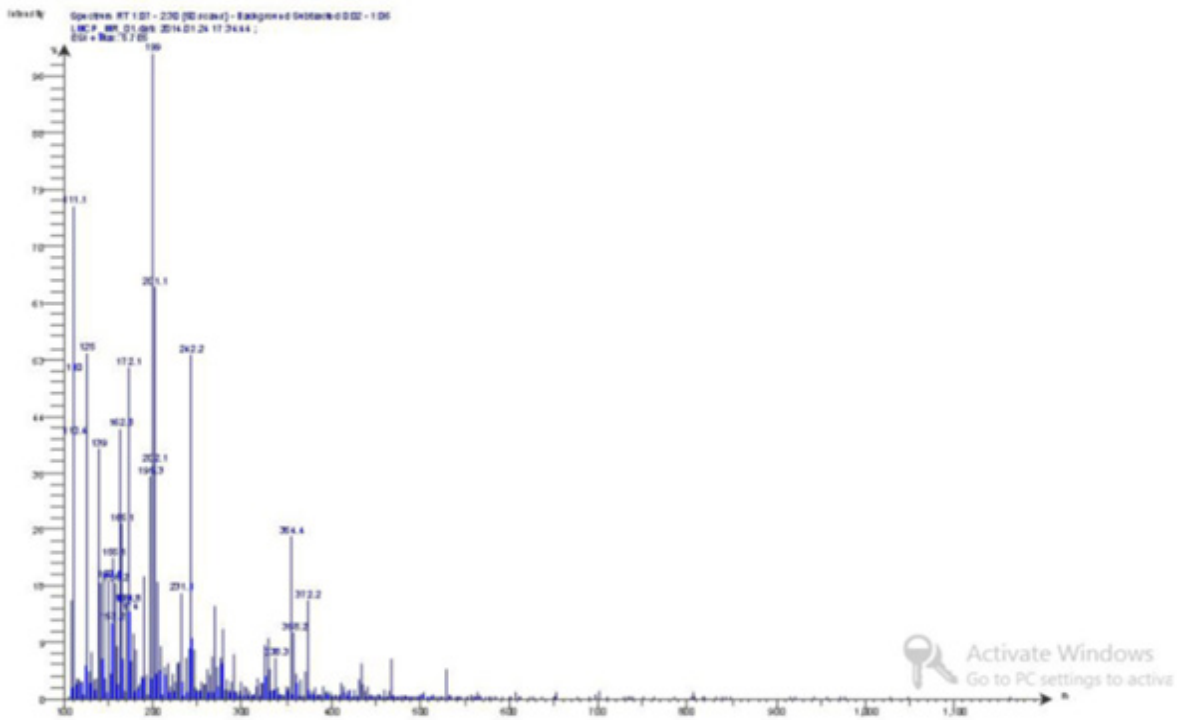


Figure 3: Mass spectra of Compound –I.

phenolic content was found as gallic acid equivalent with reference to standard curve 14.5±0.00047 mg GAE/g of dry powder and the total flavonoid content was calculated as rutin equivalent with reference to standard curve 11.2±0.0014 mg RUE/g of dry powder. Several studies have reported that flavonoids, phenolics, quinones, coumarins, alkaloids possess biological properties such as antioxidant, enzyme inhibitory, antiiflammatory, antitumor, wound healing, antiageing, and antibacterial activity.[29-33,17] Flavonoids, quinones and coumarins are among the classes of phenolic compounds. Accordingly, flavonoids have many favorable medicinal and physiological properties and are proven to exhibit antioxidative, anti-inflammatory, antitumor properties.[34] They are also known to be modulators of key cellular enzyme functions and potent inhibitors of phosphoinositide 3-kinase,[35] acetyl cholinesterase and butyl cholinesterase,[36] lipoxygenase.[37] Quinones on other hand are found to show antioxidant, anti-inflammatory and anticancer potential.[38] Coumarins show antioxidant, anti-inflammatory, anticancer, antibacterial, anticoagulant potential.[31] Pyrrolizidine alkaloids, characteristically found in plants of Boraginaceae possess antitumor property.[29] Plant sterols found to have anti-inflammatory and anticancer activity.[39] The wide-array of medicinal actions associated to the phytochemicals present in the *O. bracteatum* can be considered to be supporting the recorded ethno-medicinal uses.

Phosphomolybdate is an important *in vitro* antioxidant assay to access the total antioxidant capacity and the antioxidant potential is assessed in the conversion

of Mo (VI) to green phosphate Mo (V).[22] As shown in Table 4 TAC of A4 was found to be higher than that of other fractions, and followed by TME. Free radicals of 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) are widely used for screening of medicinal plants to investigate their antioxidant potential. The reduction of color intensity (violet color) is marked as free radical scavenging capability. As shown in Figure 6, IC_{50} of TME was found to lowest than that of other fractions, and followed by A4. In both assays marked antioxidant activity of A4 and TME was found may be due to presence of flavonoids and phenolic compounds in polar solvent. The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite that in turn diazotize with sulphanilamide acid and couple with naphthylethylenediamine, forming pink colour, which was measured at 546nm.[25] As antioxidants donate protons to the nitrite radical, the absorbance is decreased. The decrease in absorbance was used to measure the extent of nitrite radical scavenging. As shown in Figure 6 IC_{50} of A4 found to be lowest followed by A2, A1, TME and A5. The significant antioxidant potential of A4 can be attributed to flavonoid and phenolic contents, presence of alkannin and shikonin[40] that owing to their chemical nature are getting extracted in to the nonpolar solvents. Nitrite radical scavenging ability of A2 and A1 could be due to presence of alkannin and shikonin type of compounds. Overall A4 and TME are found to have good antioxidant property in all assays.

Table 3: Total phenolic and flavonoid content of the TME of *O. bracteatum*.

Extract	TPC (mgGAE/gm dry sample)	TFC (mgRU/gm dry sample)
TME	14.5±0.00047	11.2±0.0014

Values are expressed as mean ± standard deviation (SD), *n*=3

Table 4: Total Antioxidant capacity of TME and fractions of *O. bracteatum*.

Extract/Fractions	µg BHTE/mg of dry extract
TME	37.75±0.0012
A1	18.25±0.0015
A2	24.08±0.0016
A3	37.19±0.0018
A4	58.56±0.0015
A5	30.5±0.0012

Values are expressed as mean ± standard deviation (SD), *n*=3

The Brine Shrimp Lethality Assay (BSLA) was carried out to evaluate the cytotoxic potential of *O. bracteatum* against the brine shrimp *Artemia salina* nauplii. The result of BSLA is summarized in Table 5. The percent mortality of the brine shrimp nauplii was observed to be increasing with the increase in the concentration of the test solutions. Apparently, the percent mortality of the nauplii was in the order of A4≥A1≥A2>TME>A3>A5. LC_{50} value found for A4, A1, A2, and TME were 74.13µg/ml, 75.85µg/ml, 79.43µg/ml, and 131.82µg/ml respectively and for the other two fractions it was more than 500µg/ml. It was clearly noted that A4, A1 and A2 exhibited equivalent cytotoxicity that could be credited to the presence of quinones, flavonoids, etc. The positive control (Potassium dichromate), on the other hand, gave the lowest LC_{50} value of 13.63µg/ml. Plant extracts with IC_{50} less than 200ppm are considered significantly active[41] and have the potential for further investigation. The BSL assay is an effective antitumor prescreen test and may be used for reducing the cost for preliminary testing in antitumor drug discovery expeditions using comparative molecular field analysis

Table 5: LC_{50} of the extract and Fractions; and percent mortality (%) of the Brine Shrimp nauplii after 24hr exposure to the Extract and Fractions of *O. bracteatum*.

	% Mortality						
Conc (µg/ml)	1	10	100	500	1000	LC_{50}	Inference
TME	0±0.00	16.66±5.77	40.66±11.01	83.33±5.77	93.33±5.77	131.82	Cytotoxic
A1	0±0.00	16.66±5.77	60±10.0	83.33±5.77	100±0.00	75.85	Cytotoxic
A2	0±0.00	23.33±5.77	56.66±11.54	76.66±5.77	100±0.00	79.43	Cytotoxic
A3	0±0.00	3.33±5.77	23.33±5.77	36.66±5.77	53.33±11.54	575.43	Non-cytotoxic
A4	0.±0.00	16.66±5.77	66.33±11.54	83.33±5.77	100±0.00	74.13	Cytotoxic
A5	0±0.00	3.33±5.77	16.66±5.77	33.33±11.54	56.66±5.77	602.55	Non-cytotoxic
Std $K_2Cr_2O_7$	0±0.00	36.66±5.77	100±0.00	100±0.00	100±0.00	13.63	Potent Cytotoxic

Data are expressed as mean ± SD. Each sample was analyzed three times.
LC_{50} < 200 mg/L is cytotoxic

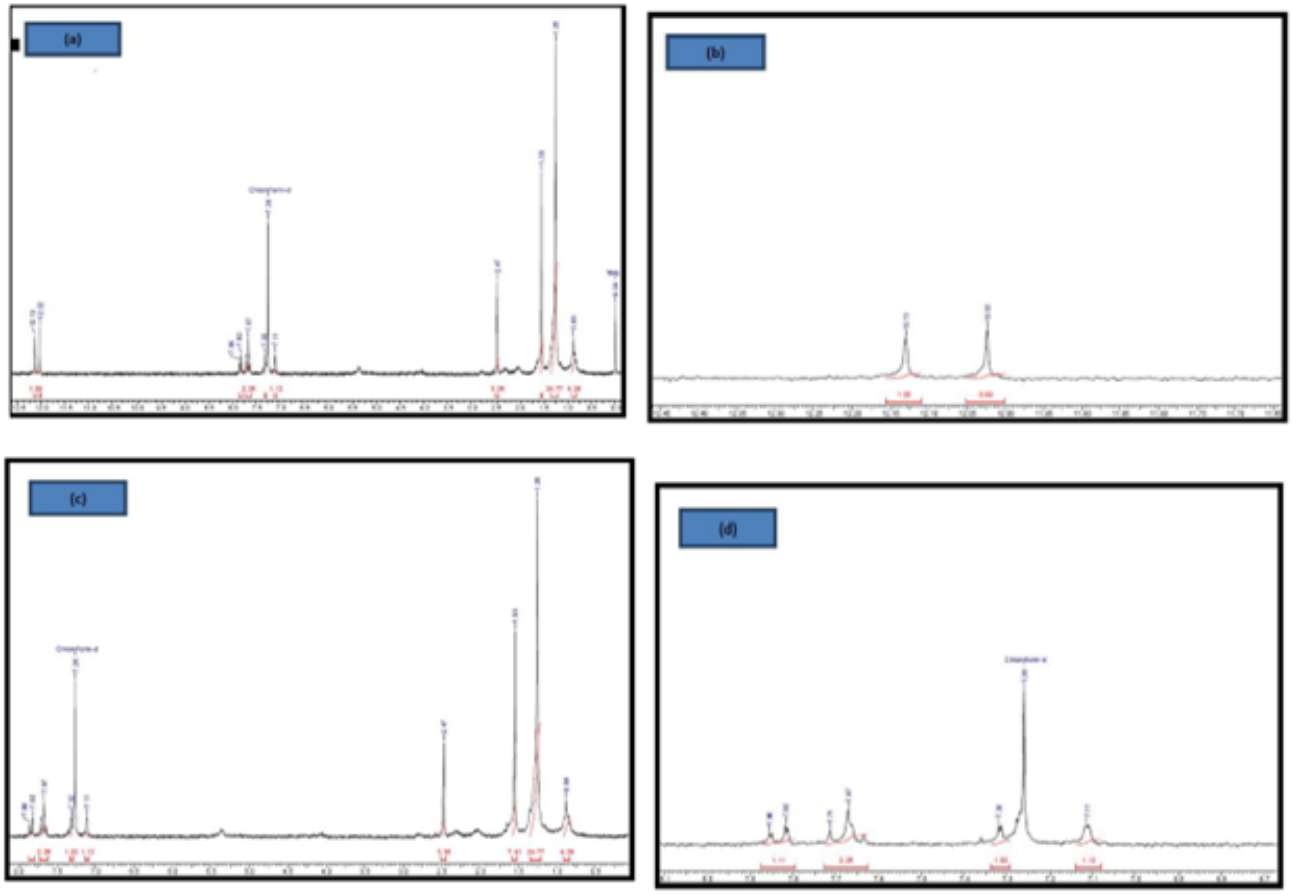


Figure 4: NMR spectra of Compound –I.

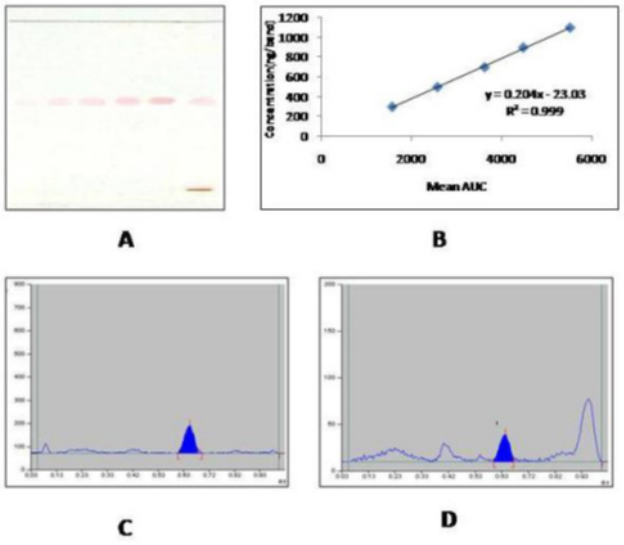


Figure 5: HPTLC study of Compound I.

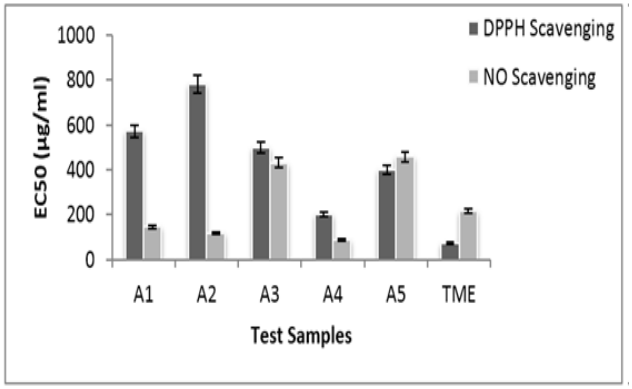


Figure 6: EC_{50} value (µg/ml) for the DPPH radical scavenging and NO scavenging activity of Extract and Fractions of *O. bracteatum.*

Error bars are standard error (n=3).

(CoMFA).[42] Positive correlation between BSLA and Human cancer cell panel (MCF-7, NCI-H460 and SF-268) have been reported, as the BSLA is limited in its predictive capacity to distinguish between strong-to-moderate and weak potency cytotoxic compounds but at finer level of discrimination for anticancer activity required the human cancer cell line.[43] Moreover, it is important to note that BSLT appears to be predictive of antifungal and pesticidal activities too.[44] The evaluations of the *in vivo* acute toxicity as well as determining the

IC_{50} values using the brine shrimp lethality test were performed in numerous studies to confirm that the brine shrimp lethality assay is useful tool for the screening of the plant extract, to predict the toxicity level.[45-47]

CONCLUSION

Structure of isolated compound-I was established was 1,7- dihydroxy-3-methyl-9H-xanthene-9-one and was found to be 0.17 ± 0.11%w/w. From the present study it was observed that the TME and A4 fraction of *O. baracteatum* wall possessed significant antioxidant potential and could be rationalized taking into account the presence of phenolics and flavonoid contents in it. A4, A1, A2 and TME also showed significant cytotoxic activity in BSL assay, which might be attributed to the presence of quinones, alkaloids, coumarins and flavonoids. It is advantageous to use the plant antioxidants in therapeutic drugs for the implications of human health as the antioxidants from natural products greatly contribute to the prevention of the progression of various diseases caused by free radicals, such as certain cancers. Thus, studies to further establish the bioactivity of *O. bracteatum* and to isolate the compounds responsible for its bioactivity is under progress.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

TME: Total Methanolic Extract; **TPC:** Total Phenolic Content; **TFC:** Total Flavonoid Content; **HPTLC:** High Performance Thin Layer Chromatography; **TAC:** Total Antioxidant Capacity; **DPPH:** 2,2 diphenyl-1-picrylhydrazyl; **BSLA:** Brine Shrimp Lethality Assay; **GAE:** Gallic Acid Equivalent; **RUE:** Rutin Equivalent; **BHTE:** Butylated Hydroxytoluene; **EC_{50}:** Effective Concentration; **LC_{50}:** Lethal Concentration; **SD:** Standard Deviation; **TLC:** Thin Layer Chromatography.

SUMMARY

The study revealed the presence of phytochemicals such as alkaloids, quinones, phytosterols, fixed oil and fats, phenolics, flavonoids, coumarins and carbohydrates in the plant. Isolated compound-I, xanthone was found to be 0.17%w/w in plant. TME and A4 fraction found to be potential antioxidant could be due to presence of phenolics and flavonoids. A4, A1, A2 and TME showed considerable cytotoxicity in BSLA, cytotoxicity of A1 and A2 are might be due to presence of xanthone, alkannine/ shikonin types of compounds. The study promising denotes *O.bracteatum* as a potential source of antioxidant and cytotoxic compounds.

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