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Original Article

Phytochemical and Pharmacological Studies of Different Extracts of Stem Bark and Leaf of *Flueggea leucopyrus* Willd.

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ABSTRACT

Background: Traditional medicinal plants are generally extracted and evaluated to identify potential sources of effective drugs. **Objective:** The present study aimed to conduct the phytochemical and pharmacological evaluation of stem bark and leaf extracts of *Flueggea leucopyrus*. **Materials and Methods:** The collected plant material was dried, powdered, and extracted separately by soxhlation with different solvents viz., petroleum ether, n-hexane, chloroform, acetone, methanol, and water. All the extracts were subjected to phytochemical evaluation. Alkaloids were extracted and characterized from the powdered sample of leaf and bark. *In vitro* antioxidant activity of the extracts was evaluated by DPPH (2, 2-diphenyl-1-picrylhydrazyl) and nitric oxide radical scavenging assay and anti-inflammatory activity by inhibition of protein denaturation and Human red blood cell (HRBC) membrane stabilization method. **Results:** Extracts were found to be either semisolid or solid with different tints of green or brown colour. The preliminary phytochemical evaluation found alkaloids, glycoside, phenolic compounds, flavonoids, carbohydrates, sterol, and saponin. On spectral evaluation, the presence of an alkaloid, Securinol-A was found in a fraction extracted from the bark. In the *in vitro* antioxidant and anti-inflammatory activity the tested samples showed a concentration-dependent rise of activity, particularly, the isolated fraction and acetone extract of stem bark revealed a significant activity. **Conclusion:** Alkaloids, flavonoids, sterols, and saponins identified in these extracts may be responsible for these biological activities. Hopefully, our *in vitro* and *in vivo* evaluations and compound-level studies in the future will reveal significant data for the development of clinically useful chemotherapeutic agents.

Key Words: *Flueggea leucopyrus*, Extraction, Phytochemical evaluation, Spectral evaluation, *In vitro* antioxidant activity, *In vitro* anti-inflammatory activity.

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INTRODUCTION

Nature represents a distinctive and extensive reservoir of phytochemicals that has inspired the development of numerous clinically validated therapeutic agents.[1,2] Phytochemicals are a dominant group of compounds otherwise known as secondary metabolites of plants including a variety of chemical entities such as isoprenoids, polyphenols, sterols, saponins etc.[3,4] These metabolites facilitate the plant growth and development by protecting them from insects, harmful UV irradiation and temperatures.[5] Phytochemicals derived from a range of traditional medicinal plants are typically extracted and evaluated to identify potential sources of effective therapeutic agents. In this context, *Flueggea leucopyrus* Willd. is selected for our research. It is a shrub that grows primarily in the wet tropical biome. It is an erect, many-branched, up to 5m tall. Have angular branchlets end in sharp spines. Leaves are alternate, obovate to elliptic, and measure up to 2.5cm long and 1.5cm wide. Male flowers are greenish-yellow and clustered in axillary fascicles, while female flowers are solitary. The male flowers have five perianth lobes, with five free stamens and a disc of five glands alternating with the stamens. The fruits are globose, about 5mm across, three-celled, and white when ripe. The seeds are trigonous, smooth, and pale brown.[6]

This plant has a long history of utilization in Ayurveda and traditional medicine, particularly within the context of Sri Lankan folk practices. The traditional medicinal system of Sri Lanka uses the leaves of *F. leucopyrus* in the treatment of cancer, boils, external ulcers, and sores. The folk medicinal system of many African and Asian countries uses various species of the genus Flueggea to treat many diseases including epilepsy, malaria, jaundice, intestinal worms, oedema, heavy menstruation, sterility, poliomyelitis, and aplastic anaemia. In Sri Lanka, the leaves of *F. leucopyrus* are used in the diet in the form of a salad or `porridge' in villages, and the fruits are consumed in India and Africa.[7]

With all this, as a part of our research on *F. leucopyrus*, the present study aimed to carry out the phytochemical and pharmacological evaluation of different extracts of stem bark and leaf of *F. leucopyrus*.

MATERIALS AND METHODS

Collection and identification of plant material

The stem bark and the leaves of the plant *Flueggea leucopyrus* Willd. was collected from Seethangoli, a place located nearer to Kasaragod town of Kerala, India. The collected material was identified and authenticated by Dr. Biju P., Assist. Professor in Dept. of Botany, Government College, Vidyanagar, Kasaragod, Kerala, India.

Powdering, extraction and preliminary phytochemical evaluation

Powdering, extraction, and preliminary phytochemical evaluation of the extracts for various

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phytoconstituents were carried out by the procedure in the published literature[8-12] with slight modification. Initially, the collected plant material, stem bark, and leaves of *F. leucopyrus* dried individually in the shade for about one week were powdered by using a mechanical grinder, and the coarse powder thus obtained was stored individually in the airtight container for extraction and further study. The successive solvent extraction of the coarse powder of stem bark and leaves (35gm) was done individually with the solvents (500ml) of ascending order of polarity viz., petroleum ether, n-hexane, chloroform, acetone, methanol, and water by soxhlet extraction procedure. After each extraction, the same dried marc was used for the subsequent extraction. Each extract was filtered, distilled off the solvent and the dried extract was obtained. Finally, the percentage yield of each extract was noted and all the extracts were subjected to preliminary phytochemical evaluation.

Extraction and isolation of alkaloids

Extraction of alkaloids from the powdered sample of both leaf and bark was done by the standard procedure.[13,14] Initially, the powdered drug was defatted with petroleum ether, marc thus obtained was extracted with methanol, and the crude methanolic extract was evaporated and concentrated. The residue was treated with 1% HCl and partitioned with diethyl ether. The aqueous-acid phase was separated and made alkaline with ammonium hydroxide at controlled pH (7.5). Then it was partitioned with chloroform. Both chloroform and aqueous phases were tested for the presence of alkaloids. The chloroform phase containing alkaloid was confirmed by TLC using the solvent system Chloroform: methanol (9:1) and detected by Dragendorff's reagent.

About 200gm of silica gel was mixed with a sufficient quantity of chloroform, and the slurry formed filled almost 1/2th volume of the column (45cm length; 2cm Dia) without air bubbles. The column was gently tapped to ensure the uniform settling of the solid. The excess solvent was drained off and when the level was 1 inch above the solid phase, the tap was closed. A few drops of triethylamine were added and the column was run with chloroform twice. Then continued with the loading of the sample. Initially, the column was eluted with chloroform (100%) followed by a graded mixture of Chloroform: Methanol (0%, 0.25%, 0.50%, 0.75%). The elution was monitored by TLC (Silica gel G; visualization: Dragendroff spraying reagent as orange spot). Each time 10 ml were collected and the identical elutes (TLC monitored) were combined, concentrated, and kept in a desiccator. The isolated compound was characterized by different spectroscopic evaluations viz., ^{13}C NMR, ^{1}H NMR, Mass, and IR.

In vitro antioxidant activity

In vitro antioxidant evaluation of all the extracts was done by different approaches such as DPPH (2, 2-diphenyl-1-picrylhydrazyl) and nitric oxide radical scavenging assay.[12,15,16]

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay

The reaction mixture contains 2.5ml of selected test extracts in different concentrations (50, 100, 150, and 200µg/ml) and 1ml of alcoholic solution of DPPH (0.3mM). It was kept in 30min. incubation in the dark at room temperature. Then, the absorbance of the reaction mixtures was measured at 518nm, using ethanol as the blank, DPPH in ethanol as the control, and ascorbic acid as the standard control. The inhibition percentage of DPPH radical by the tests was identified by

$$\% \text{ inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

The percentage of inhibition against concentration was plotted as a graph. The IC_{50} values of the samples were assessed from the regression equation of the graph.

Nitric oxide radical scavenging assay

The reaction mixture (3ml) was prepared by mixing sodium nitroprusside (10mM; 2ml) in phosphate buffer (pH 7.3; 0.5ml) and selected test extracts in different concentrations (50, 100, 150, and 200µg/ml). After the addition of 1ml of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) to the reaction mixture, it was kept at 25°C (in front of 25W tungsten lamp) for 3h. The nitric oxide radical thus formed interacted with oxygen to produce nitrite ion which was measured spectrophotometrically (540nm). Normal and standard controls were prepared. Ascorbic acid was employed as the standard control. The percentage inhibition nitric oxide radical formation was determined by

$$\% \text{ inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

In vitro anti-inflammatory activity

The anti-inflammatory activity of the selected extracts was evaluated *in vitro* by inhibition of protein denaturation and Human red blood cell (HRBC) membrane stabilization method.[17-21]

Inhibition of protein denaturation

The reaction mixture (0.5ml) contains bovine serum albumin (0.45ml; 5% aq. solution) and plant extracts (0.05ml) in different concentrations (50, 100, 150, 200µg/ml). 1N HCl was used to adjust the pH of the reaction mixture to 6.3. Distilled water and ibuprofen were used as normal control and standard control respectively. The reaction mixture was incubated at 37°C for 20min and then heated to 57°C for 20min. After cooling, 2.5ml phosphate buffer saline (pH 6.3) was added to each tube and the absorbance was measured spectrophotometrically (660 nm). The percentage inhibition of protein denaturation was calculated by

$$\% \text{ inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

HRBC membrane stabilization method

Fresh whole human blood (10ml) obtained from the healthy volunteer who did not use any NSAIDs for two weeks was centrifuged (3000rpm for 10min), washed with normal saline (3 times), and reconstituted as 10%v/v suspension in normal saline. For heat-induced haemolysis assay, equal volume (1ml) of test extracts in different concentrations (50, 100, 150, and 200µg/ml) and HRBC suspension (10%v/v) were mixed and incubated at 56°C for 30min. The mixture was then cooled and centrifuged (2500rpm for 5min) to obtain the supernatant. The absorbance of collected supernatants was measured spectrophotometrically (560nm) and from this, the percentage

$$\% \text{ inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

RESULTS AND DISCUSSION

In the present study, after the taxonomical identification of collected stem bark and leaves of *F. leucopyrus* by the botanist, the collected material was powdered and extracted by soxhlation with different solvents viz., petroleum ether, n-hexane, chloroform, acetone, methanol, and water. The colour, consistency, and percentage yield of the extracts were analyzed. Results showed that all the extracts were semisolid in consistency except the methanol and aqueous extract of stem bark which were solid. In case of colour, each extract was found either in different tints of green colour viz., light green, yellowish green, and dark green, or brown colour viz., brown, dark brown, reddish brown, and wine red which are illustrated in Table 1. In case of

Table 1: Nature and percentage yield of different extracts of *F. leucopyrus*.

Solvent used for extraction	Colour of the extract		Consistency of the extract		% yield of the extract (%w/w)	
	Bark	Leaf	Bark	Leaf	Bark	Leaf
Petroleum ether	Light green	Green	Semisolid	Semisolid	1.56	3.36
n-hexane	Yellowish green	Yellowish green	Semisolid	Semisolid	0.30	0. 84
Chloroform	Dark green	Dark green	Semisolid	Semisolid	1.18	7.36
Acetone	brown	Brown	Semisolid	Semisolid	6.02	13.48
Methanol	Wine red	Brown	Solid	Semisolid	7.80	5.52
Water	Reddish brown	Dark brown	Solid	Semisolid	9.40	6.56

Table 2: Qualitative phytochemical analysis of different extracts of *F. leucopyrus*.

No	Test for Phytoconstituents	Pet. Ether extract		n-Hexane extract		$CHCl_3$ extract		Acetone extract		Methanol extract		Aqueous extract	
		L	B	L	B	L	B	L	B	L	B	L	B
1.	**Alkaloids**												
a.	Mayer's test	-	-	-	-	+	++	-	-	+	+	+	+
b.	Wagner's test	-	-	-	-	+	++	-	-	+	+	+	+
c.	Hager's test	-	-	-	-	+	++	-	-	+	+	+	+
d.	Dragendorff's test	-	-	-	-	+	++	-	-	+	+	+	+
2.	**Glycosides**												
a.	Legal's test	-	-	-	-	-	-	+	+	+	+	+	+
b.	Baljet's test	-	-	-	-	-	-	+	+	+	+	+	+
c.	Borntrager's test	-	-	-	-	-	-	+	+	+	+	+	+
d.	Modified Borntragers' test	-	-	-	-	-	-	-	-	-	-	-	-
3.	**Phenolics**												
a.	Ferric chloride test	-	-	-	-	-	-	++	+	++	++	+	+
b.	lead acetate test	-	-	-	-	-	-	++	+	++	++	+	+
c.	Gelatin test	-	-	-	-	-	-	++	+	++	++	+	+
4.	**Flavones& Flavonoids**												
a.	Aqueous NaOH test	-	-	-	-	-	-	++	+	++	+	+	+
b.	Ammonia test	-	-	-	-	-	-	++	+	++	+	+	+
5.	**Carbohydrates**												
a	Molish's test	-	-	-	-	-	-	-	-	+	+	+	+
b.	Benedict's test	-	-	-	-	-	-	-	-	+	+	+	+
c.	Fehling's test	-	-	-	-	-	-	-	-	+	+	+	+
6.	**Proteins & Amino acids**												
a.	Millon's test	-	-	-	-	-	-	-	-	-	-	-	-
b.	Biuret test	-	-	-	-	-	-	-	-	-	-	-	-
c.	Ninhydrin test	-	-	-	-	-	-	-	-	-	-	-	-
7.	**Terpenoids**												
a.	Salkowski's test	-	-	-	-	-	-	-	-	-	-	-	-
8.	**Sterols**												
a.	Libermann Buchard's test	+	+	+	+	-	-	+	+	+	+	+	+
b.	Salkowski's test	+	+	+	+	-	-	+	+	+	+	+	+
9.	**Saponins**												
a.	Foams test/ Froth test	-	-	-	-	-	-	+	+	+	+	+	++
b.	Hemolysis test	-	-	-	-	-	-	+	+	+	+	+	++
10.	**Gum & mucilage**	-	-	-	-	-	-	+	+	+	+	+	+

L : Leaf; B : Bark; (+) : presence of active constituents; (++) : significant presence of active constituents; (-) : absence of active constituents

percentage yield, the acetone extract of leaf gave the highest percentage of yield (13.48%w/w), next to that the aqueous and the methanol extract of bark yielded 9.40%w/w and 7.80%w/w respectively.

Table 2 shows the results of qualitative phytochemical analysis of these extracts for the identification of various phytoconstituents such as alkaloids, glycosides, phenolics, flavonoids, carbohydrates, proteins and amino acids, terpenoids sterols, saponins, etc. The presence of alkaloids, glycosides, phenolic compounds, flavonoids, carbohydrates, sterol, and saponin was found in the tested extracts. The chloroform, methanol, and aqueous extracts of leaf and bark gave positive results in t`and aqueous extracts of both leaf and bark showed the presence of carbohydrates. Proteins and amino acids and terpenoids were not found in the tests. The presence of sterol was found in all the tested extracts except chloroform.

In this study, an alkaloidal compound was isolated from the powdered material of stem bark and leaf. The alkaloids appeared as orange spots on TLC analysis (Figure 1). Based on the yield obtained, the fraction extracted from the bark of *F. leucopyrus* was used for column chromatography. Elution carried out with Chloroform: Methanol (0.75%) resulted in a single spot on TLC (Chloroform: Methanol;

8.5:1.5). This fraction was collected and evaporated to yield a brown-coloured mass which was designated as expected alkaloidal compound and subjected to different spectral analysis. The other elutes appeared in brown resinous masses and were not processed further. The physical characterization data of the isolated compound is presented in Table 3 & Figure 1.

The IR spectral data of the isolated compound is shown in Figure 2 & Table 4. The ^{13}C NMR and ^{1}H NMR data are illustrated in Table 5 and Figure 3 & 4. Data of the mass spectrum showed M+1 peak (Figure 5) and the isolated compound was found as "Securinol-A" (Figure 6) and has a molecular formula of "$C_{13}H_{17}NO_3$" with a molecular weight of 235.0.

Table 3: Physical characters of the isolated compound.

Parameter	Observation
Colour	Brown
R_f value	0.73 [Solvent system; chloroform: Methanol (8.5:1.5)].
Colour of the spot	Orange (Spraying reagent: Dragendorff's reagent).
Solubility	Chloroform, DMSO
Melting point	155° C

Table 4: IR Spectral data of the isolated compound.

No	Type of Vibration	Observed Value (cm^{-1})
1	C=O Stretching	1740
2	-OH stretching	2965
3	C-O-C stretching	1633
4	C-N Stretching	1456
5	C=C Stretching	1220

Table 5: ^{13}C NMR and ^{1}H NMR spectral data of isolated compound.

Spectral method		Group assigned	Peak region
^{13}C NMR		C=CH	120.43
		R-COO-R	143.90
		$-CH_2$	32.81-32.08
		-CH	35.37-36.08
		-C	54.94-59.73
	Type of peak	**Group assigned**	**Peak region**
^{1}H NMR	Multiplet	-NH	1.39-1.90
	Multiplet	-C-H	5.673-5.796
	Multiplet	-C=CH	6.489-6.782
	Singlet	-OH highly deshielded proton	7.134

Table 6: Antioxidant activity of test extracts and extracted fraction by DPPH assay.

Conc. (µg/ml)	% inhibition by extracts										
	Std. (A. A)	Test extracts									
		1		2		3		4		5	
		B	L	B	L	B	L	B	L	B	L
50	60.55	40.07	46.18	41.22	47.70	50.50	41.60	42.80	42.45	55.21	42.36
100	70.61	55.59	49.49	48.22	55.59	59.60	50.25	50.12	50.50	60.06	50.38
150	75.44	63.74	61.32	55.09	65.90	70.10	63.23	59.16	55.97	72.64	60.05
200	82.21	72.51	71.50	70.36	74.30	74.81	72.39	64.25	70.86	76.94	71.88
IC_{50}	78.49	110.86	113.70	122.78	104.11	97.19	113.83	123.56	119.68	91.5	116.02

1- Chloroform; 2-Acetone; 3-Methanol; 4-Water; 5-Extracted fraction; A. A- Ascorbic acid; B-Bark; L-Leaf

Figure 1: TLC analysis in the extraction and isolation of alkaloid from *F. leucopyrus*.

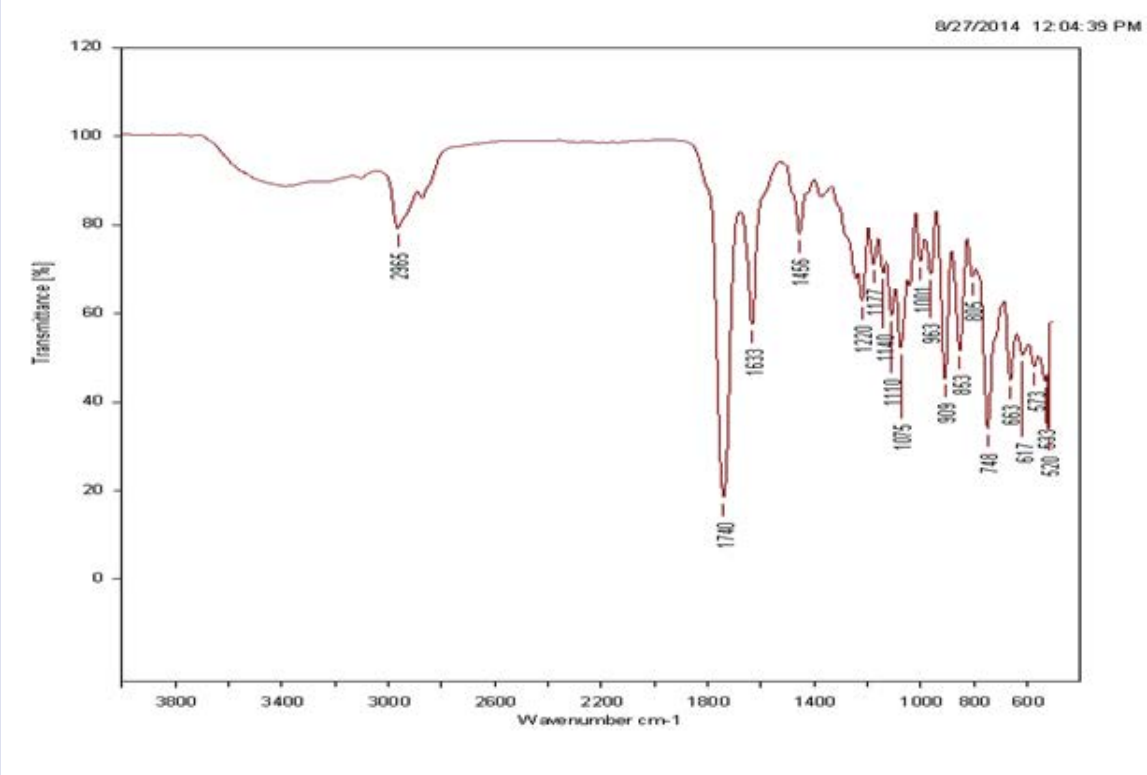

Figure 2: IR spectrum of the isolated compound.

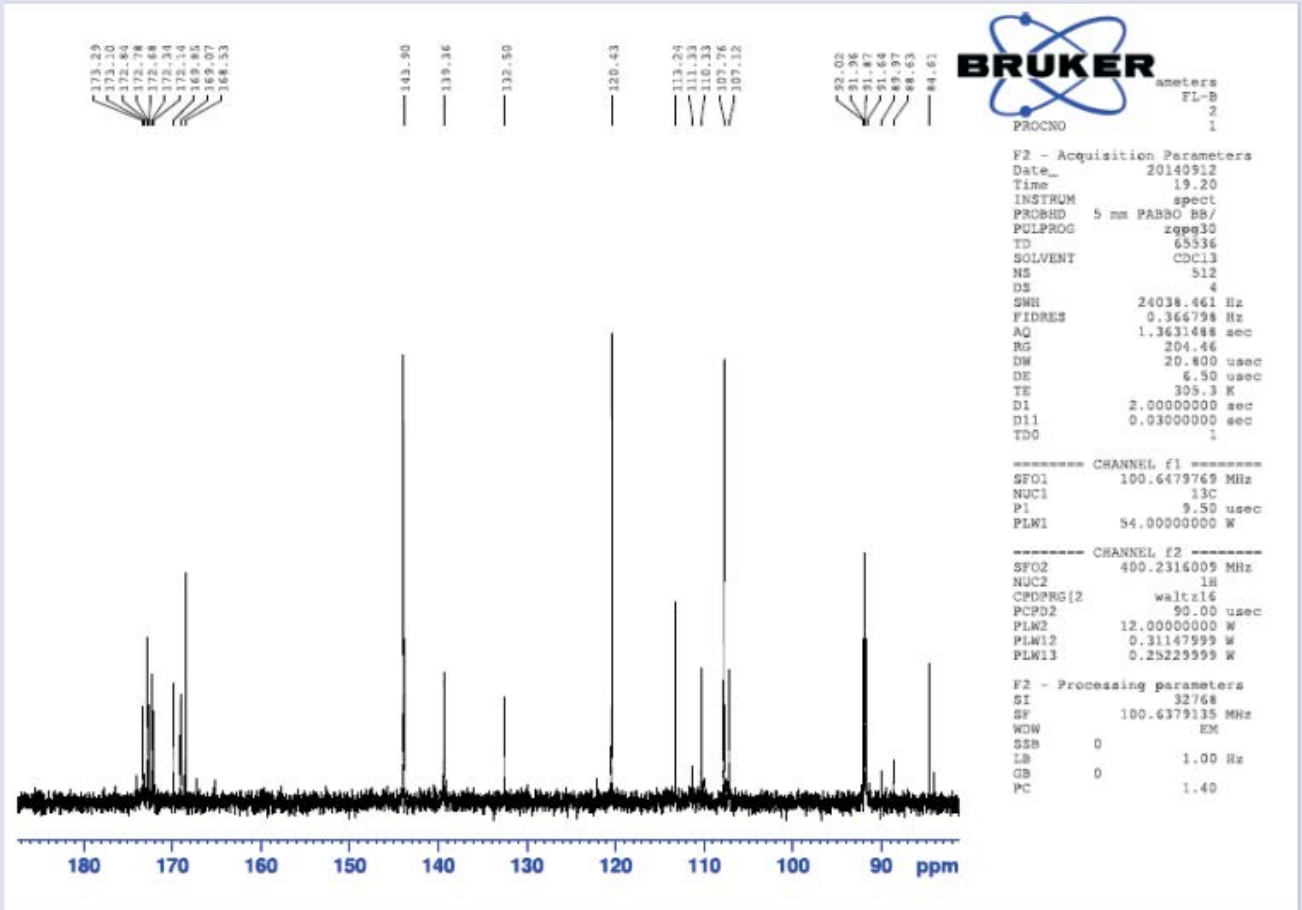

Figure 3: ^{13}C NMR Spectrum of the isolated compound.

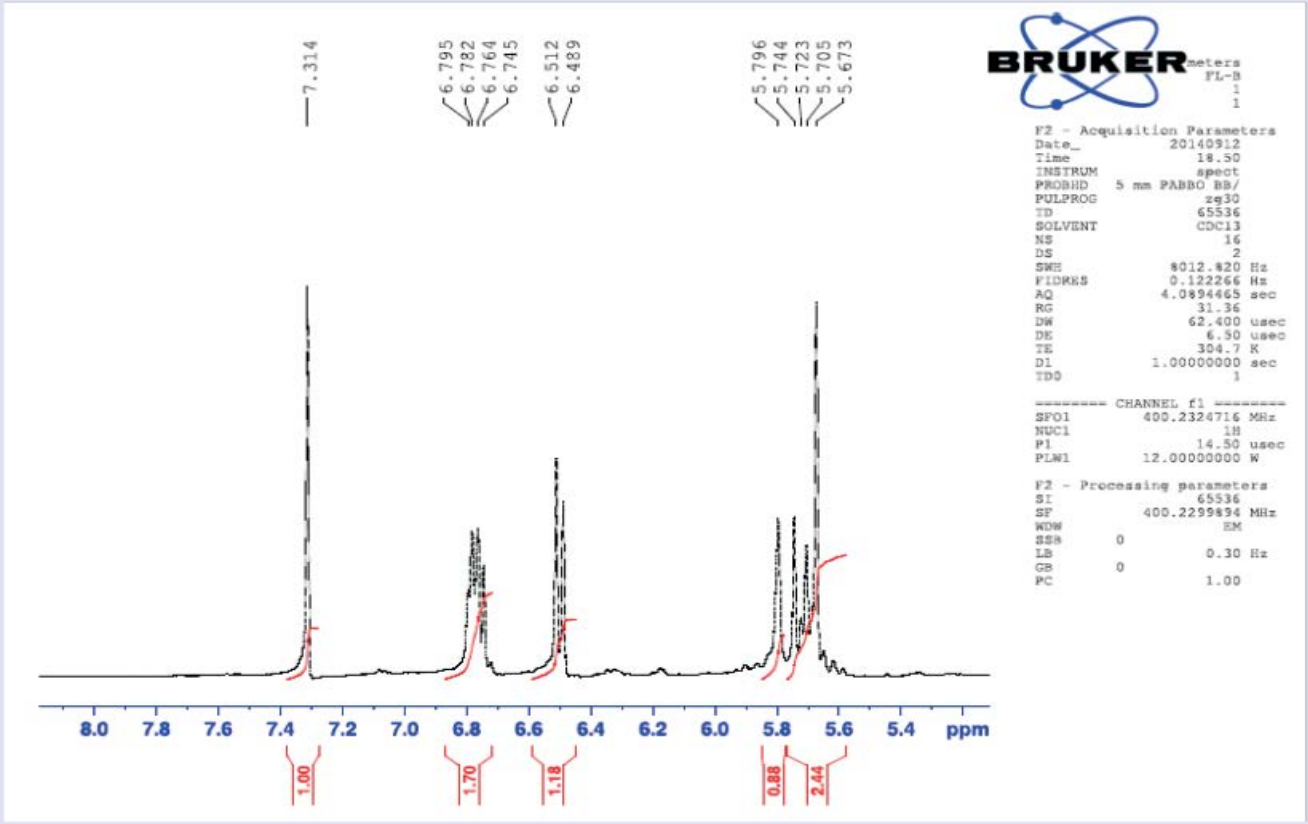

Figure 4: [1]H NMR Spectrum of the isolated compound.

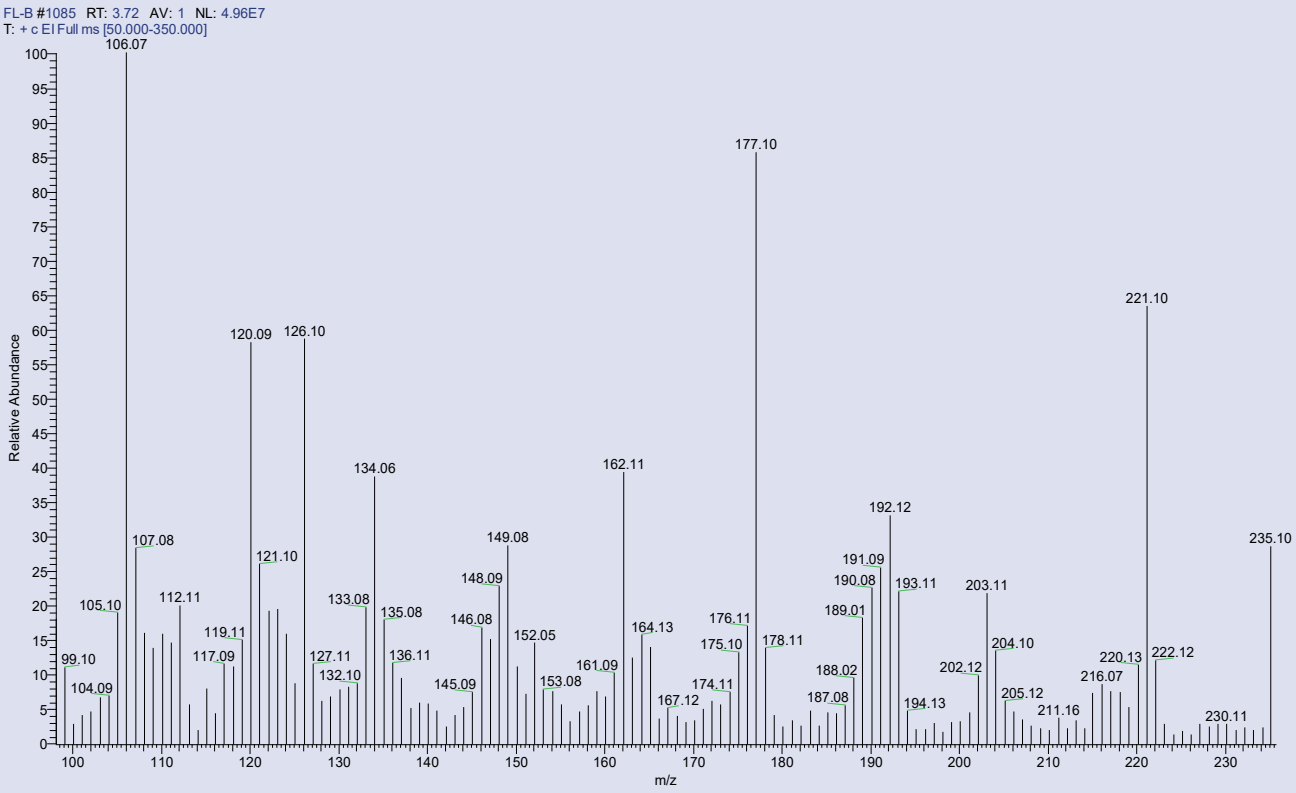

Figure 5: Mass Spectrum of the isolated compound.

Table 7: Antioxidant activity of test extracts and extracted fraction by nitric oxide radical scavenging assay.

Conc. (µg/ml)	% inhibition by extracts										
	Std. (A. A)	Test extracts									
		1		2		3		4		5	
		B	L	B	L	B	L	B	L	B	L
50	69.69	36.36	40.40	67.68	64.65	51.52	48.48	34.30	31.31	31.31	28.28
100	75.70	50.50	47.47	74.75	71.72	62.63	55.56	46.50	47.47	57.57	54.54
150	81.82	66.60	59.59	78.79	79.79	68.69	64.65	53.50	55.56	71.72	65.66
200	82.88	70.00	68.69	79.79	81.82	75.75	74.75	65.66	63.64	78.76	74.75
IC_{50}	66.37	115.76	121.69	107.95	113.85	95.01	104.08	133.26	134.36	70.21	73.28

A.A-Ascorbic acid; 1-Chloroform; 2-Acetone; 3-Methanol; 4-Water; 5-Extracted fraction; B-Bark; L-Leaf

Table 8: *In vitro* anti-inflammatory evaluation of test extracts and extracted fraction by protein denaturation inhibition method.

Conc. (µg/ml)	% inhibition by extracts						
	Std. (Ibu.)	Test extracts					
		1		2		3	
		B	L	B	L	B	L
50	36.34	43.98	29.40	37.19	26.70	44.06	38.50
100	58.95	63.66	39.51	44.14	37.04	59.88	48.15
150	73.69	70.60	61.27	61.58	45.14	68.52	62.27
200	78.09	74.77	72.84	73.38	70.91	75.77	72.07

Ibu-Ibuprofen; 1- Acetone; 2-Methanol; 3- Extracted fraction; B-Bark; L-Leaf

Table 9: *In vitro* anti-inflammatory evaluation of test extracts and extracted fraction by HRBC membrane stabilization method.

Conc. (µg/ml)	% protection by extracts						
	Std. (Ibu.)	Test extracts					
		1		2		3	
		B	L	B	L	B	L
50	49.28	52.39	40.67	53.35	46.65	53.83	53.59
100	64.59	67.46	53.59	61.48	57.18	64.83	60.77
150	70.10	70.33	64.59	67.22	62.92	71.05	68.42
200	78.95	75.60	72.25	73.68	70.33	76.56	72.73

Ibu-Ibuprofen; 1- Acetone; 2-Methanol; 3- Extracted fraction; B-Bark; L-Leaf

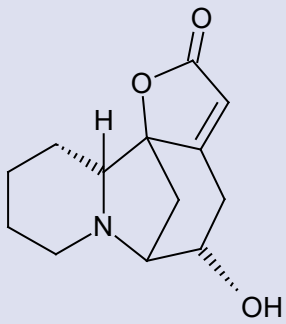

Figure 6: Structural representation of the isolated compound Securinol A.

Based on the results of the preliminary phytochemical evaluation, the chloroform, acetone, methanol, and aqueous extract of both bark and leaf were selected for the *in vitro* antioxidant evaluation, and the extracted fraction was also subjected to this evaluation. The results of *in vitro* antioxidant evaluation by DPPH assay are shown in Table 6. In this assay, all the tested extracts showed a concentration-dependent rise in activity. Generally, all the tested samples revealed the highest percentage of inhibition at the concentration of 200µg/ml, the highest one employed for the evaluation. Among all the test extracts, the extracted fraction of bark showed the highest percentage (76.94%) of inhibition at the concentration of 200µg/ml which is comparable with the score of ascorbic acid (82.21%). IC_{50} value was calculated for each extract and standard from the graph which is shown in Table 6. A similar type of result, the concentration-dependent rise of activity was found in the nitric oxide radical scavenging assay, another method employed for the evaluation of antioxidant activity (Table 7). The flavonoids and sterols are well known for their anti-oxidant activity.[23-25] These phytochemicals were found in the preliminary phytochemical evaluation of the present study which may be responsible for the antioxidant activity reported.

Based on the results of the preliminary phytochemical evaluation and in vitro antioxidant activity, acetone methanol and extracted fraction from both bark and leaf were selected for the *in vitro* anti-inflammatory

evaluation by two methods such as inhibition of protein denaturation and stabilization of HRBC membrane. In both these evaluations also, all the tested extracts showed activity, importantly, the bark extracts showed significant activity compared with leaf extracts. All the extracts showed a concentration-dependent rise of activity and the maximum activity was found on 200µg/ml test concentration. The extracted fraction of bark showed significant activity compared with other tested extracts in both the methods employed. It showed significant activity in terms of inhibition of protein denaturation by 75.77% and protection of HRBC membrane by 76.56%. Next to that, the acetone extract of bark showed activity of 74.77% and 75.60% respectively (Tables 8 & 9). The phytochemicals such as flavonoids, terpenoids, saponins, tannins, alkaloids, anthraquinones, and essential oils play an important role in the treatment of inflammatory diseases.[26] Alkaloids, flavonoids, and saponins found in the tested extracts may be responsible for the anti-inflammatory activity found in the present study.

CONCLUSION

In the present study, the leaf and bark of the *Flueggea leucopyrus* were collected, authenticated, and dried and the powdered material was subjected to successive solvent extraction by soxhlation using different solvents such as petroleum ether, n-hexane, chloroform, acetone, methanol, and water. Phytochemical studies of these extracts showed the presence of alkaloids, glycosides, phenolic compounds, flavonoids, carbohydrates, sterol, and saponins. In addition, alkaloids were extracted from the powdered material. The extracted compound was found to be more in bark and showed better separation when subjected to thin-layer chromatography. Further, the fraction was purified by column chromatography and designated as the expected compound. The isolated compound was analysed by different spectral methods such as ^{13}C NMR, ^{1}H NMR, Mass, and IR. The data obtained from the spectral studies shows the presence of a true alkaloid "Securinol-A". The in vitro antioxidant and anti-inflammatory activity on the selected extracts and the isolated fraction showed a significant activity particularly, the isolated fraction of stem bark and acetone extract of bark. Alkaloids, flavonoids, sterols, and saponins identified in these extracts may be responsible for these biological activity. Hopefully, our *in vitro* and *in vivo* evaluations and compound-level studies in the future will reveal significant data for the development of clinically useful chemotherapeutic agents.

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CONFLICTS OF INTEREST

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