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Phytochemical and Pharmacological Investigation of Lespedesea Gerardiana

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Abstract: *The World Health Organization (WHO) is giving more prominence to promoting traditional medicine, particularly, in third-world countries. The global market of medicinal plants is estimated at over US$ 60.0 billion which is expected to grow up to US$ 5.0 trillion by the year 2050. In Pakistan, the bill for the import of medicinal plants was worth US$ 31.0 million, whereas the export of medicinal plants could hardly reach US$ 6.0 million. In the world, 30% of the pharmaceutical preparations are manufactured from plants. Keeping in view the potential of the global and local market, we conducted our current research work entitled Phytochemical and pharmacological investigations of Lespedesea gerardiana The present dissertation comprises: Pharmacological/Biological activities of crude methanolic extract (Crd. MeOH Ext.), various fractions and phytochemical study of L. gerardiana. Crd. MeOH Ext. and various fractions of the plant were investigated for various pharmacological/biological activities including, Brine-shrimp lethality, Haemagglutination, and Insecticidal and Antioxidant assay.*

Key Words: Antioxidant Activity, β-Sitosterol, Ursolic Acid, and Stigmasterol

Introduction

RIGVEDA' (1600–4500 BC) refers to the plants which were originally recorded for usage as medicine. Plants have been utilised as medications since prehistoric times due to their medicinal powers (Bako, et al., 2005). In addition, several scientists and many Muslim scientists, including Al-Ghafiqi, Ibn Sina, Al-Dinawari, and Al-Dimashqi, made substantial contributions to the discipline of botany and collected plants for their studies from a range of sources. These scientists explored the medical use of plants.

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Ayurvedic medicinal books like Susruta Samhita and Charaka Samhita mention using around 700 plants derived from minerals and animal sources (Gilani, 2005). The poll claims that every ancient civilization has repeated the discovery of medicinal plants.

All civilizations, including Chinese, Greek, Indian, Roman, Egyptian, and Pakistani, have carried on these customs throughout history. European traditions have also played a significant role in the advancement of herbalism.

Plants are an excellent source of medicinal materials, particularly in traditional medicine where they are used to treat a wide range of illnesses (Bako, et al., 2005). Ancient Greeks practised the use of herbal remedies. These days, research indicates that this plant has compounds with anti-inflammatory and blood-clotting qualities (de Almeida et al., 2023)

We have chosen L. gerardiana, a shrub leaf used as fodder that belongs to the Fabaceae family (Yahara et al 2013). Legumes' roots serve as an anchor for bacteria that fix nitrogen from the air into the soil, which can then be absorbed by subsequent plants and lower the need for fertiliser inputs. Members of the fabaceae Desmodium styracifolium and Desmodium gyrans have historically been used to cure a variety of illnesses, including rheumatism, diarrhoea, pyrexia, wounds, malaria, cough, hepatitis, and hemoptysis. Ćurić et al., 2023)

The fabaceae family, which comprises over 19400 species and 730 genera, is the third largest group of flowering plants and is distributed worldwide. (Oldfield & Gardiner, 2023; Yahara et al., 2013)

Thus far, this genus has yielded descriptions of 100 leguminous species, which comprise herbs, shrubs, and semi-shrubs (Allen & Allen, 1981; Yao 2002). Additionally, 70 species have been discovered in China. Although nothing is known about the micro symbionts of this genus, they create root nodules (Gempo et al., 2024).

Material and Method

Haemagglutination Activity

Procedure

Phosphate buffer was prepared by combining 0.47g/50ml of Na2HPO4 and 0.453g/50ml of KH2PO4 in distilled water at a ratio of 3:7. To prepare the stock solution, 1 mg of test samples were dissolved in 1 ml of DMSO. Six dilutions, namely 1:12, 1:10, 1:8, 1:6, 1:4, and 1:2, were prepared in phosphate buffer using stock solutions. On the day of the trial, blood was drawn from healthy individuals and centrifuged.

Phosphate buffer The suspension of 2% RBC was ready. One millilitre of the test sample was taken from each dilution in a test tube, and one millilitre of the fresh RBC suspension was added. The tubes were then incubated for thirty minutes at 37 degrees Celsius. The tubes were centrifuged after incubation in order to see agglutination. Rough granules and soft buttons formed, indicating both positive and negative consequences (Liu et al., 2023)

Diphenyl Picryl Hydrazyl Radical-Scavenging Activity

Although oxygen is necessary for life, scientists are also finding that these vital components have a role in human disease and ageing. During metabolism, oxygen releases free radicals that enter the cell, disrupt molecular structure, and ultimately cause cellular destruction. Ageing and numerous health issues are caused by such effects. Antioxidants function as defence mechanisms for the cell's machinery, scavenging free radicals from damage. Antioxidants prevent cellular damage, are ingested by food, or are produced by the body spontaneously (Perveen et al., 2023).

Researchers are looking at how antioxidant levels in food and dietary supplements affect the body's ability to produce free radicals. Plant samples were subjected to a little modification of Blois (1958) [41]'s approach to measure their antioxidant activity. The capacity of the relevant plant extract's electrons or hydrogen atoms against 2, 2-diphenyl-1-picryl hydrazyl (DPPH) was

measured using UV spectrophotometry at 517 nm. 1ml DPPH solution was prepared in methanol.1 ml from that solution was put into 3 ml of sample solutions in ethanol (containing 20-500 µg). A standard drug (querceitin) was used as positive control separately, containing no sample (Takeda et al., 2023). The rate of absorbance was deliberate at 517 nm (SP-3000 PLUS Spectrophotometer, Optima, Japan) after 30 min. A rise in the DPPH radical scavenging activity was shown by a decrease in the DPPH solution absorbance. % RSA (percent radical scavenging activities) or scavenging of free radicals by DPPH was measured through the below formula:

$$\% \text{ RSA} = 100 \times \frac{\text{Control abs - sample abs}}{\text{Control abs}}$$

It was acknowledged that three assays were conducted, and the outcomes are presented as mean values standard deviations.

Brine Shrimp Lethality Bioassay

Every year, around 1.2 million new cases are diagnosed; the prostate, lung, breast, rectum, and colon account for half of these instances. Cancer can strike anyone at any age, but it most frequently strikes those over 50. The brine shrimp lethality bioassay requires modest amounts of test material (2–20 mg), is affordable, simple to learn, and can be completed in 24 hours. For the first time, pacitaxol and taxol were separated from the Himalayan Yew tree's bark (Pohan et al., 2023).

The test samples' cytotoxic impact on brine shrimp eggs was conducted in accordance with the protocol as described in. To facilitate hatching, commercial salt and deionized water were used to create artificial seawater in a plastic plate. 50 mg of eggs were added to the bigger and smaller, unevenly divided plastic dishes that were light- and dark-coloured, respectively. For 48 hours, the setup was maintained at 25 oC to allow the eggs to mature. Nauplii were harvested using a Pasteur pipette following a 48-hour maturation period. As a stock solution, two millilitres of organic solvent were mixed with twenty milligrams of test materials. (Banurekha et al., 2023)

Three vials were filled with stock solutions at concentrations of 5, 50, and 500 µl each. The vials were left in the hood for a full hour or overnight to allow the organic solvent to evaporate. Ten larvae and one millilitre of seawater were added to each vial. A vial's absolute volume was set at 5 millilitres using seawater. Vials were incubated for twenty-four hours at 26 1 C below illumination. A cytotoxic medication called etoposide (7.4625 µg/ml) was utilised as the positive control and an organic solvent as the negative control. The number of brine prawns that survived the 24-hour incubation period under a magnifying glass was counted.

Insecticidal Activity

Test Samples Preparation

To prepare the stock solutions, the test samples (200 mg) were dissolved in 3 ml of methanol.

The aforementioned pests were raised in plastic bottles under controlled laboratory conditions, including temperature and humidity, using a sterile medium (breeding). For the purpose of the experiments, insects of the same age and size were used.

The application of the contact toxicity procedure determined the insecticidal assay. The essay includes the steps below. Filter sheets were cut to match the size of sterilised Petri plates on the first day. Filter sheets were placed into Petri plates, and then test sample stock solutions were added with a micropipette. (Jiang et al., 2023)

The petri plates were exposed to methanol for a whole night. Ten new, similar-sized insects from each of the three species were selected and moved (to the labelled plates) on the second day using a clean brush (Zhong et al, 2023). For one day, plates were incubated at 27 C with 50% relative humidity in a growth chamber.

$$\text{Percentage death} = 100 - \frac{\text{No. of insects alive in test}}{\quad} \times 100$$

No. of insects alive in control Permethrin (235.9µg/cm2) was utilised as a positive control and methanol as a negative one.

Results

Haemagglutination Activity

At all dilutions (1:2, 1:4, 1:8, 1:16), the results showed no activity against human erythrocytes belonging to any blood type (A+ive, B+ive, AB+ive, O+ive, A-ive, B-ive, AB-ive, and O-ive). The findings showed that phytolectins are absent from plants.

Diphenyl Picryl Hydrazine Radical-Scavenging Activity

Using a DPPH radical scavenging experiment, the antioxidant efficacy of Crd. MeOH Ext. L. gerardiana extracts and different fractions were evaluated

According to the data, at a concentration of 20µg/ml Crd. The lowest activity was seen in MeOH Ext. (16%), n-hexane (32.44%), $CHCl_3$, and EtOAc (9.5% and 18.0%), respectively.

The Crd. MeOH Ext. exhibited poor activity (19.3%) and n-hexane exhibited low activity (34.07%) at a concentration of 40µg/ml. In a similar vein, the activities of $CHCl_3$ and EtOAc were modest (13.8% and 19.5%, respectively). The Crd. MeOH Ext. exhibited low activity (20.0%) at a concentration of 60 µg/ml, whereas the remaining fractions—n-hexane, $CHCl_3$, and EtOAc—also displayed low activity (36.1%), 14.4%, and 24.9%, respectively. At 80µg/ml, there was minimal activity for Crd. MeOH Ext. (21.0%), n-hexane (37.7%), $CHCl_3$ (15.9%), and EtOAc (33.7%). Comparably little activity was seen at concentrations of 100, 250, and 500 µg/ml for Crd. MeOH Ext. and other L. gerardiana fractions.

Table 1

Antioxidant activity of Crd. MeOH Ext. and various fractions of L. gerardiana

Conc. µg/ml	Percent activity			
	Crd. MeOH Ext.	*n*-hexane	$CHCl_3$	EtOAc
20	16.0	32.44	9.5	18.0
40	19.3	34.07	13.8	19.5
60	20.0	36.1	14.4	24.9
80	21.0	37.7	15.9	33.7
100	24.0	39.6	18.0	34.0
250	26.0	40.9	21.6	35.0
500	27.0	41.5	26.5	36.0

Diphenyl Picryl Hydrazine Radical-Scavenging Activity

According to the data, at a concentration of 20µg/ml Crd. The results indicated modest activity in MeOH Ext. (16%), n-hexane (32.44%), $CHCl_3$, and EtOAc (9.5% and 18.0%), respectively. When the Crd is concentrated to 40µg/ml. Low activity was seen in MeOH Ext. (19.3%) and n-hexane (34.07%). In a similar vein, the activities of $CHCl_3$ and EtOAc were modest (13.8% and 19.5%, respectively). The Crd. MeOH Ext. exhibited low activity (20.0%) at a concentration of 60 µg/ml, whereas the remaining fractions—n-hexane, $CHCl_3$, and EtOAc—also displayed low activity (36.1%), 14.4%, and 24.9%, respectively. At 80µg/ml, there was minimal activity for Crd. MeOH Ext. (21.0%), n-hexane (37.7%), $CHCl_3$ (15.9%), and EtOAc (33.7%). Comparably little activity was seen at concentrations of 100, 250, and 500 µg/ml for Crd. MeOH Ext. and other L. gerardiana fractions.

Table 2

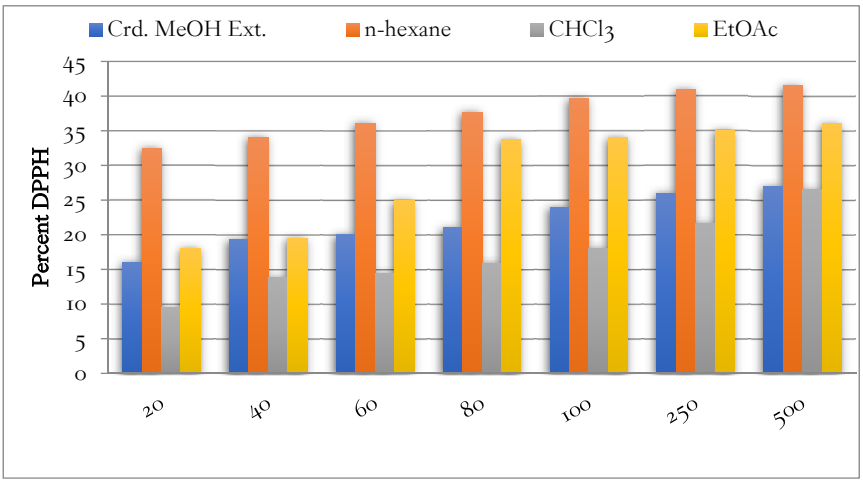
Antioxidant activity of Crd. MeOH Ext. and various fractions of L. gerardiana

Conc. µg/ml	Percent activity			
	Crd. MeOH Ext.	*n*-hexane	$CHCl_3$	EtOAc
20	16.0	32.44	9.5	18.0
40	19.3	34.07	13.8	19.5
60	20.0	36.1	14.4	24.9
80	21.0	37.7	15.9	33.7
100	24.0	39.6	18.0	34.0
250	26.0	40.9	21.6	35.0
500	27.0	41.5	26.5	36.0

*Standard drug *Querceitin as control*

Figure 1

Antioxidant activity of Crd. MeOH Ext. and various fractions of L. gerardiana



Brine Shrimp Lethality Bioassay

The test samples' cytotoxicity was evaluated using the Artemiasalina cytotoxic assay [52]. The results of this exercise are shown in Figure 3.14 and Table 3.7. According to the data, at 1000µg/ml, the Crd. MeOH Ext. exhibited modest cytotoxicity (13.33%). The cytotoxicity levels were 3.33% and 10% at 100 µg/ml and 10 µg/ml, respectively. The n-hexane fraction was shown to be harmless at concentrations of 1000, 100, and 10 µg/ml, in that order. At 1000µg/ml, the CHCl3 fraction displayed a lethality of 26.66%. There was 23.33% and 23.33% toxicity at 100 and 10µg/ml, respectively. At 1000, 100, and 10 µg/ml, there was no mortality in the EtOAc fraction. According to the aqueous fraction data, 26% of the test samples died at 1000 µg/ml, 27% at 100 µg/ml, and 28% at 10 µg/ml.

Table 3

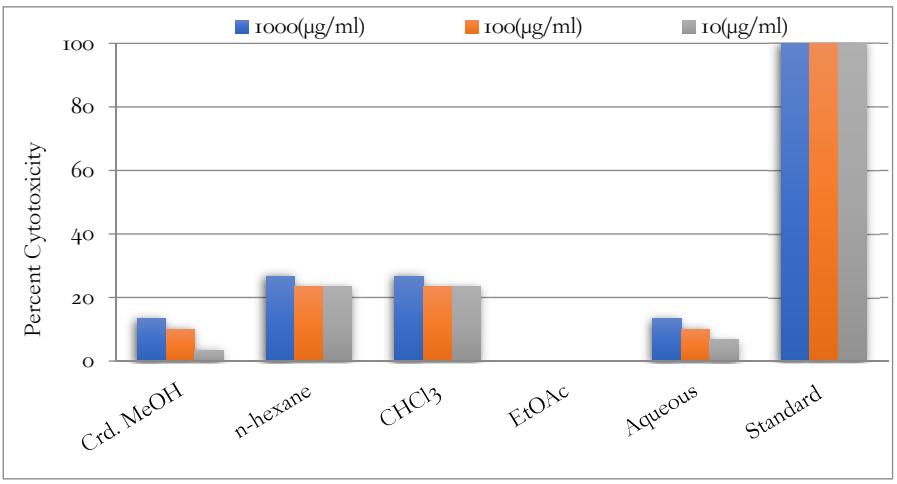
Brine Shrimp cytotoxicity of Crd. MeOH Ext. and fractions of L. gerardiana

		Crd. MeOH Ext		*n*-hexane		$CHCl_3$		EtOAc		Aqueous		Standard drugs	
Dose (µg/ml)	Control (No of Shrimp)	Survivors	% killed	Survivors	% killed	Survivors	% killed	Survivors	% killed	Survivors	% killed	Survivors	% killed
1000	30	26	13.33	30	0	22	26.66	30	0	26	13.33	0	100
100	30	27	10	30	0	23	23.33	30	0	27	10	0	100
10	30	29	3.33	30	0	23	23.33	30	0	28	6.66	0	100

*A standard drug * Etopside at a concentration of 7.4625 µg/ml*

Figure 2

Brine shrimp cytotoxicity of Crd. MeOH. Ext. and various fractions of L.gerardiana



Insecticidal Activity

The primary problem limiting crop productivity is the removal of sources such as synthetically generated pesticides, which lead to resistance and have a detrimental effect on crop and environmental health [53]. In order to replace the harmful insecticides, we must look for natural insecticides that are also environmentally beneficial. As a model approach, we employed R. dominica, T. castaneum, and T. granarium to track the insecticidal activity of plant parts. The findings are shown in Fig. 3.15 and reported in Table 3.8.

T. castaneum was not present in the test samples. The n-hexane $CHCl_3$ and EtOAc fractions demonstrated low activity of 40, 20, and 20% against R. dominica, whilst the remaining test samples exhibited no activity. Low activity (20%) was produced by the Crd. MeOH Ext., $CHCl_3$, n-hexane, and EtOAc. Against T. granarium, the aqueous fraction had no activity.

Table 4

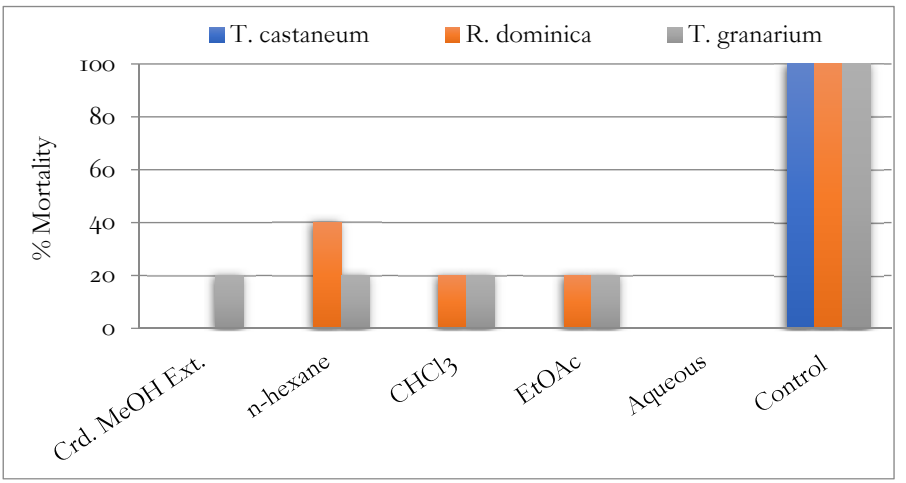
Insecticidal activity of Crd. MeOH Ext. and various fractions of L. gerardiana.

Name of insect	Percent Mortality						
	+ive Control	-ive Control	Crd. MeOH Ext.	*n*-hexane	$CHCl_3$	EtOAc	Aqueous
T. castaneum	100	0	0	0	0	0	0
R. dominica	100	0	0	40	20	20	0
T. granarium	100	0	20	20	20	20	0

A standard drug Permethrine (235.9 µg/cm² concentration) positive control

Figure 3

Insecticidal activity of Crd. MeOH. Ext. and various fractions of L .gerardiana



Phytochemical Investigations

Ursolic Acid (01)

Compound 01 was extracted as colourless needles from the plant's CHCl3 fraction. The IR spectra showed absorption for tri-substituted double bonds (1635 and 820 cm-1), OH (3510 cm-1), and carbonyl (1697 cm -1). Using HRMS, the compound's chemical formula was determined to be C30H48O3, indicating [M]+ at m/z 456.3599 (Calc d. for C30H48O3, 456.3603).

Compound 01's 1H-NMR spectra showed five tertiary methyl at δ = 0.91, 1.05, 1.08, 1.25, and 1.27. The ursane skeleton is indicated by two secondary methyl at δ: 0.99 (3H, d, J=6.4 Hz) and 1.06 (3H, d, J=6.6 Hz). At δ: 3.19 (1H, dd, J=3.5 Hz), the carbinylic proton is evident. Compound 01 revealed the presence of six quaternary, seven methane, nine methylene, and seven methyl carbon atoms at the 13C-NMR spectrum (BB and DEPT). The compound's physical and spectral studies closely matched ursolic acid reports found in the literature. Additionally, Gymnocolea inflata was reported to have the chemical.

Characterization of Compound

Physical state Colorless needles from EtOH (5.8mg)

R*f*: 0.38 ($CHCl_3$: Acetone, 7:3)

M.P: 278-280°C.

UVλ_{max}(MeOH) 220 nm

IRν_{max} cm^{-1}.820, 1635, 1697, 1705, 3050, 3541.

HREI-MS (*m/z*) 442.2314 (observed), $C_{30}H_{50}O_2$, 442.2311 (calcd.).

EI-MS *m/z* (peak %) 442 (M^+, 5.1), 425 (M^+-OH, 8.1), 424 (M^+-H_2O, 15), 409 (M^+

H_2O-CH_3, 2.0), 407 (1.2), 234 (100), 216 (34), 201 (4.5), 189 (4.3), 175 (3.4).

^{1}H-NMR (300 MHz, $CDCl_3$) δ:4.18(2H,mH-28), 5.22(1H,m,H-12), 3.39 (1H, m, H

3α), 1.21 (3H, s, CH_3), 0. 98, 0.95, 0.88, 0.87, 0.78 and 0.81 (3H, each,s,CH_3).

^{13}C-NMR (100MHz $CDCl_3$) δ: 24.0 (C-30),22.4 (C-29), 176.2 (C-28),24.5 (C-27),17.2 (C-26), 15.9 (C-25), 15.4 (C-24), 24.0 (C-23),37.0 (C-22),27.5 (C-21), 30.3 (C-20), 30.5 (C-19),55.2 (C-18),47.9 (C-17),23.5 (C-16), 29.4 (C-15),42.0 (C-14),138.7 (C-13), 125.8 (C-12), 23.9 (C-11), 37.1 (C-10), 47.4 (C-9), 39.6 (C-8), 33.2 (C-7), 18.3 (C-6), 52.4 (C-5),79.1 (C-3), 27.4 (C-2), 38.5 (C-1).

Stigmasterol

Compound 02 was separated from the plant's chloroform fraction. After detecting a [M]+ at m/z 412.3920, the chemical formula was identified as calculated C29H48O by HRMS. The infrared spectrum reveals that oxygen is hydroxyl (3432 cm-1). The mass spectrum displayed the features of the sterol fragmentation model.

The compounds' 1H-NMR spectra fully match the information for stigmasterol [60]. Signals for two tertiary methyl groups were seen at δ: 0.84 and 0.65 in the spectra, and for two multiplets of carbinylic proton, at δ: 3.28.

The availability of 29 carbon signals for 3 quaternary carbon, 11 methine, 9 methylene, and 6 methyl atoms was revealed in the compound 02, 13C-NMR (DEPT and BB) spectrum. The compound's current data and the published literature demonstrated perfect agreement with those for stigmasterol.

Characterization of Compound

Physical state: Crystalized from MeOH (20 mg).

M.P:143-145°C

IR (KBR) ν_{max}(cm^{-1}):2304, 2901, 1641.

EIMS(rel. Int. %) *m/z*:95 (21), 107 (18),119 (10),135 (9),145 (19),161 (14),213 (18),273 (12),303 (21),329 (25),396 (19),399(15), 414 (100).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.92 (3H, s, CH_3-19), 0.83 (3H, d, $J_{26,25}$ = 6.5 Hz, CH_3-26), 0.88 (3H, d, $J_{21,20}$ = 6.5 Hz, CH_3-21),0.81 (3H, d, $J_{27,25}$ = 6.5 Hz, CH_3-27),0.77 (3H, t, $J_{29,28}$ = 7.0 Hz, CH_3-29), 5.32 (1H, m, H-3α),0.63 (3H, s, CH_3-18).

^{13}C-NMR ($CDCl_3$, 100 MHz)δ: 11.9 (C-29),23.1 (C-28),19.8 (C-27), 18.8 (C-26),26.2 (C-25),50.4 (C-24),29.3 (C-23),34.0 (C-22),19.1 (C-21),36.3 (C-20),19.4 (C-19), 11.9 (C-18), 56.2 (C-17),28.2 (C-16),24.3 (C-15),56.8 (C-14), 42.6 (C-13), 40.3 (C-12),21.1 (C-11),36.6 (C-10),50.8 (C-9),35.6 (C-8), 32.1 (C-7),33.0 (C-6), 140.9 (C-5), 42.4 (C-4), 121.9 (C-3), 31.8 (C-2), 37.3 (C-1).

β-Sitosterol

A [M]+ was seen inside the EIMS complex at m/z 414. Similar to this, at m/z 414.3857, a [M]+ was seen in the HR-EIMS molecule, which was confirmed by using the chemical formula C29H50O from calculation 414.3861.

In the infrared spectra, hydroxyl bands were seen at 3450 cm-1 while signals for tri-substituted double bonds were seen at 3050, 1650, and 815 cm-1. The latter two ions investigated the presence of five degrees of unsaturated position in sterols [62].

The sterol molecule was represented by Compound (03) spectra 1H-NMR.

Owing to quaternary CH3-19 and 18, respectively, two singlets were observed assimilating for 3-H at δ: 0.63 and 0.92. Primary CH3-29 was responsible for a triplet integrate for 3H on δ 0.77 (J29,28 = 7.0 Hz), whereas secondary CH3-21, 27 and 26 were responsible for three doublets at δ; 0.88 (J21,20 = 6.5 Hz), 0.83 (J27,25 = 6.5 Hz), and 0.81 (J26,25 = 6.5 Hz) assimilating for 3H. Olefinic signals combined for the 1H signal were scattered to the H-6 double bond at δ 5.32. The signal integrating for 1H showed a typical chemical shift and splitting pattern at δ 3.36 in relation to the H-3β and H-3α hydroxyl tasks.

When the reported literature was compared to the chemical (03) NMR spectroscopic data, the result was Sitosterol.

Characterization of Compound

Physical state: Colorless crystals (16 mg)

M.P:171 – 172°C.

IR ($CHCl_3$) $V_{max}cm^{-1}$:1648 (C=C), 3432 (OH).

EIMS *m/z* (rel. int. %) $[M]^+$:270 (24), 273 (30),300 (67),301 (18),327 (60),351 (71),369 (35),379 (27),394 (20),396 (12),412 (8).

HREIMS *m/z* 412.3920 (calcd. for $C_{29}H_{48}O_3$ 412.3926).

^{1}H-NMR ($CDCl_3$, 400MHz) δ: 5.15 (1H, dd, J = 15.2, 8.4 Hz, H-22), 0.80 (3H, s, Me-19),3.28 (1H, m, H-3), 5.33 (1H, m, H-6), 0.90 (3H, d, J= 6.5 Hz, Me-21), 0.84 (3H, t, J = 7.0 Hz, Me-29), 0.81 (3H, d, J = 6.5 Hz, Me-27), 5.02 (1H, dd, J = 15.2, 8.6 Hz, H-23),0.83 (3H, d, J = 6.6 Hz, Me-26),0.65 (3H, s, Me-18).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 12.0(C-29), 25.4(C-28),129.4(C-23),138.4(C-22), 21.2(C-27),19.0(C-26),32.0(C-25), 51.3(C-24), 21.1(C-21),40.5(C-20),19.4(C-19), 12.4(C-18), 56.0(C-17),28.9(C-16), 24.4(C-15),57.0(C14),42.5(C-13),39.7(C-12),21.0(C-11), 36.6(C-10),50.3(C-9), 32.2(C-8), 121.7(C-6),140.9(C-5),42.2(C-4), 71.9(C-3), 31.9(C-2),37.5(C-1).

References

Allen, O. N., & Allen, E. K. (1981). *The Leguminosae is a source book of characteristics, uses, and nodulation*. Univ of Wisconsin Press.

Bako, S. P., Bakfur, M. J., John, I., & Bala, E. I. (2005). Ethnomedicinal and phytochemical profile of some savanna plant species in Nigeria. *International Journal of Botany*.

Banurekha, J., Sangeetha, M., Mahendran, C., & Muruganatham, S. V. (2024). Phytochemical Evaluation of Different Solvent Mediated Extracts and Brine Shrimp Lethality Bioassay of Psychotria octosulcata WA Talbot. *International Journal of Nanoscience*, *23*(2), 2350064. https://doi.org/10.1142/S021958lX23500643

Ćurić, M., & Spiridonov, V. (2023). *Brief General Historical Overview. In History of Meteorology* (pp. 15-29). Cham: Springer Nature Switzerland.

De Almeida, I. G., & de Fátima Rosa, M. (2023). The Mesopotamian civilisation: An overview. *Images, Perceptions and Productions in and of Antiquity*, 138.

Gempo, N., Yeshi, K., Jamtsho, T., Jamtsho, L., & Wangchuk, P. (2024). Development of quality control parameters for two Bhutanese medicinal plants (Aster flaccidus Bunge and Aster diplostephioides (DC.) Benth. ex CB Clarke) using traditional and modern pharmacognostical platforms. *Heliyon*, *10*(3). https://doi.org/10.1016/j.heliyon.2024.e24969

Gilani, A. H. (2005). Trends in ethnopharmacology. *Journal of Ethnopharmacology*, *100*(1-2), 43-49.

Jiang, K., Chen, Z., Zang, Y., Shi, Y., Shang, C., Jiao, X., & Gao, X. (2023). Functional characterization of Vip3Aa from Bacillus thuringiensis reveals the contributions of specific domains to its insecticidal activity. *Journal of Biological Chemistry*, *299*(3). https://doi.org/10.1016/j.jbc.2023.103000

Liu, Y. T., & Wang, C. H. (2023). Loss of Haemagglutination Activity of an Infectious Bronchitis Virus after Attenuation. *Taiwan Veterinary Journal*, *48*(03n04), 75-83. https://doi.org/10.1142/S168264852350004X

Oldfield, S., & Gardiner, L. (2023). Flowering Plants. *The Living Planet: The State of the World's Wildlife*, 18.

Perveen, S., Iqbal, S., Wajid, S., & Choudhary, M. I. (2023). 1, 1-Diphenyl-2-picrylhydrazyl and superoxide anion radical scavenging 1 activities of heterocyclic 2-oxo-1, 2, 3, 4-tetrahydropyrimidines. *arXiv preprint arXiv:2310.10063*. https://doi.org/10.4103/0974-8490.72323

Pohan, D. J., Marantuan, R. S., & Djojosaputro, M. (2023). Toxicity test of strong drug using the BSLT (brine shrimp lethality test) method. *International Journal of Health Sciences and Research*, *13*(2), 203-209. https://doi.org/10.13005/ojc/360618

Takeda, S., Ahhmed, A. M., Sogawa, K., Mouri, S., Kaneko, S., Sakata, R., & Mizunoya, W. (2023). Antioxidant activity of venison subjected to in vitro cooking and gastrointestinal digestion and isolation of its 2, 2-diphenyl-1-picrylhydrazyl radical scavenging peptides. *Animal Science Journal*, *94*(1), e13870. https://doi.org/10.1111/asj.13870

Yahara, T., Javadi, F., Onoda, Y., de Queiroz, L. P., Faith, D. P., Prado, D. E., & Nkonki, T. (2013). Global legume diversity assessment: concepts, key indicators, and strategies. *Taxon*, *62*(2), 249-266. https://doi.org/10.12705/622.12

Yao, Z. Y., Kan, F. L., Wang, E. T., Wei, G. H., & Chen, W. X. (2002). Characterization of rhizobia that nodulate legume species of the genus Lespedeza and description of Bradyrhizobium yuanmingense sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *52*(6), 2219-2230. https://doi.org/10.1099/00207713-52-6-2219

Zhong, L. K., Sun, X. P., Han, L., Tan, C. X., Weng, J. Q., Xu, T. M., & Liu, X. H. (2023). Design, Synthesis, Insecticidal Activity, and SAR of Aryl Isoxazoline Derivatives Containing Pyrazole-5-carboxamide Motif. *Journal of Agricultural and Food Chemistry*, *71*(40), 14458-14470. https://doi.org/10.1002/ps.7897