ISSN 2664-7354

Volume: 1, Issue: 2
Page: 295-305
2019

International Journal of Academic Research in Business, Arts and Science (IJARBAS.COM)

Evaluation of Phytochemical Constituents, Analgesic and Anti-Inflammatory Efficacy of the N-Butanol Partitioned Portion of the Aerial Parts of Laggera Aurita Linn. In Albino Rats.

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Abstract:

People are aware that medicinal plants have been identified and used throughout the history of human being, this is due to their capability to synthesize a wide variety of chemical compounds that perform important biological functions. The plant Laggera aurita (Asteraceae) is a shrub usually found along the pathways, in farmlands and around houses, nearby yards and is reported to have vast healing powers on conditions as such constipation, dyspepsia, inflammation, and aiding of wound healing etc. This paper seeks to scrutinize the analgesic and anti-inflammatory effects of the n-butanol partitioned part of the aerial portion of Laggera aurita Linn. using formalin induced pain and egg albumin persuaded inflammation in rats and as well as to evaluate its phytoconstituents. Actually the results of the phytochemical evaluation of the n-Butanol partitioned portion revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, terpenoids and tannins with the most significant effects on inflammation and pain at 700 mg/kg in the both models.

IJARBAS
Accepted 15 August 2019
Published 16 August 2019
DOI: 10.5281/zenodo.3369849

Keywords: Analgesic, Anti-inflammatory, Phytochemistry, Laggera aurita, n-butanol,

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Introduction

The use and search for drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural-products chemists are combing the earth for phytochemicals and leads that could be developed for treatment of various diseases. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants (Isagedeghi, 2013). More than two thirds of the world's plant species, at least 35,000 of which are estimated to have medicinal value come from the developing countries. At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants (IENICA, 2000–2005).

Laggera aurita is a widely used medicinal plant in African countries like Nigeria, Senegal, Tanzania and Ghana. The leaves are the part of the plant reported to be most commonly used for medicinal purposes, although the plant can be used whole or pulped up. The main indications were to treat rheumatic pain, to heal cuts, and bruises, as an enema, as an insect repellant, to cure constipation and dysentery and as a last resort to phagedenic and chronic ulcers. The powdered plant or infusions are used for dyspepsia and indigestion in Nigeria (Burkill, 1985).

Pain is an unpleasant sensory and emotional experience caused by real or potential injury or damage to the body or described in terms of such damage. Scientists believe that pain evolved in the animal kingdom as a valuable three-part warning system. First, it warns of injury. Second, pain protects against further injury by causing a reflexive withdrawal from the source of injury. Finally, pain leads to a period of reduced activity, enabling injuries to heal more efficiently (Besson, 1999). While, an inflammation is the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells or irritants (Ferrero-Miliani et al., 2007). It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue (Cotran, 1998).

Sequel to and earlier publication in chemistry research journal on the phytoconstituents, analgesic and anti-inflammatory effects of the crude and n-hexane defatted portion of the aerial part of Laggera aurita Linn. using formalin induced pain and egg albumin induced inflammation in rats, which revealed that the crude has significant activity, bioassay partitioning was carried to examine the phytoconstituents, analgesic and anti- inflammatory effects of the n-butanol partitioned portion using similar protocol.

Methodology

Sample Collection, Preparation and Extraction

The sample was collected around Maiduguri metropolis, Borno state, and was identified by a taxonomist at the department of Biological Sciences University of Maiduguri. The sample was dried under shade until a constant weight was obtained. The aerial parts were pulverized using wooden mortar and pestle from which 1000 g of the powdered materials was exhaustively extracted with 80% ethanol using soxhlet apparatus. The extract was concentrated under reduced pressure and then defatted with n-hexane. The concentrate was further fractionated with chloroform, ethylacetate and n-butanol in aqueous system. The resulting mass of the n-butanol was subjected to phytochemical and pharmacological evaluations using standard procedures.

Phytochemical Evaluation of the n-butanol partitioned portion of the aerial part of Laggera aurita

Phytochemical evaluations were carried out on the n-butanol partitioned portion of the aerial part of Laggera aurita as using standard procedures as reported by ; (Silva et al., 1998); (Evans, 2002); (Vishnoi, 1979); (Sofowora, 1993); (Brain and Tuner, 1975); (Markham, 1982) for alkaloids, anthraquinone, flovonoids, tannins, saponin and phlobotannins e.t.c

Experimental animals:

Fifty (50) Wister albino rats of both sexes weighing between 150-230 g were used for this study. The animals were kept and maintained under natural condition at the Animals House in the Department of Biochemistry, University of Maiduguri and fed with Vital Poultry feeds (grower mash) and water ad libitum.

Anti- inflammatory Activity of the extracts: Egg Albumin-Induced Rat Paw Oedema

Five (5) groups of five (5) rats were administered n-butanol partitioned portion of the aerial part of Laggera aurita (350, 700 or 1400 mg/kg i.p), piroxicam (20 mg/kg i.p) or normal saline as control (0.5 ml/kg) 1 hour before the induction of inflammation. Acute inflammation was produced by the sub-planter administration of 0.1 ml fresh egg albumin into the right hind paw of each rat 1hour after administration of respective doses of the extracts. The paw volumes were measured at 0 min to 120 min, taking the readings at 20 minutes intervals (Akah and Nnambie, 1994), After the egg albumin administration, Vanier caliper was used to measure the average volume of the right hind paw of each rat from four readings which did not deviate more than 3% (Ascongelem et al., 2004).

The anti-inflammatory effect of the partitioned portion was calculated using the following equation:

$$\text{percentage inhibition of oedema} = \left(\frac{vc - vt}{vc}\right) X100$$

Where vc = control represent the groups administered normal saline (negative control) and vt=treatment represents the groups administered the portion and piroxicam (Gupta et al., 2005).

Analgesic Studies of n-butanol Partitioned Portion: Formalin-Induced Pain in Rats

Twenty five (25) adult rats of both sexes were grouped randomly into five (5) groups of five (5) rats. Rats in group A were administered normal saline (0.2 ml i.p.) only; rats in group B were administered the standard analgesic drug (piroxicam) 20 mg/kg bd.wt while those in group C, D and E were administered: 350, 700 and 1400 mg/kg bd.wt of the n-butanol partitioned portion extract respectively. All the treatments were done intraperitoneally. Thirty (30) minutes after administration of the portion, each rat in all groups was injected with 20 µl of 1% formalin at plantar surface of the left paw and immediately placed in a transparent plastic chamber (Hunsker and Hole 1987). The rats were observed for the first 5

minutes and then from 20-30 minutes after formalin injection. The time spent in licking the injected paw was recorded during these periods to represent pain perception.

The analgesic effect of the extract was calculated using the following equation:

$$\text{percentage inhibition of pain} = \left(\frac{vc - vt}{vc}\right) X100$$

Where vc = control represent the groups administered normal saline (negative control) and vt = treatment represents the groups administered the portion and piroxicam (Gupta et al., 2005).

Results

Table 1 shows the results of the phytochemical evaluation of the n-butanol partitioned portion of the aerial part Laggera aurita Linn.

Phytochemical Evaluation	N-Butanol Partitioned Portion
Alkaloids	+
Anthraquinone	-
Cardiac glycosides	+
Flavonoids	+
Phlobatannins	-
Saponins	+
Tannins	+
Terpenoids	+

Key:

(+)Present, (–) Not detected

Table 2: Shows the results of the formalin induced pain in rats of the n-butanol partitioned portion of the aerial part Laggera aurita Linn.

Values along same column differently superscripted differ significantly (P<0.05)

Dose (mg/kg bd.wt.)	No. of Paw Licking (Mean ± SEM)/ Percentage Inhibition (%)			
	0 -5 min.	(%)	20-30 min.	(%)
350	59.40±11.57[a]	(27.91)	45.40±12.99[a]	(63.85)
700	43.00±6.89[ad]	(47.82)	38.20±3.40[ac]	(74.64)
1400	42.20±6.97[adf]	(48.79)	53.20±4.04[ace]	(64.67)
Piroxicam 20	23.80±6.31[bdf]	(71.12)	38.20±7.35[ace]	(74.63)
N. Saline 0.2 ml	82.4±4.26[ceg]		150.60±11.35[b]	

Table 3 Shows the results of the egg albumin induced inflammation in rats of the n-butanol Partitioned Portion of the aerial part Laggera aurita Linn.

Values along same column differently superscripted differ significantly (P<0.05)

Dose(mg/kg)	TIME (min.)/ Oedema Level (Mean ± SEM) mm						
	0	20	40	60	80	100	120
350	0.00±0.00	3.64±0.17^{a}	3.52±0.18^{a}	3.28±0.12^{a}	2.92±0.23^{a}	2.60±0.23^{a}	2.50±0.14^{a}
		(6.67)	(9.74)	(21.90)	(35.11)	(42.22)	(45.18)
700	0.00±0.00	2.64±0.26^{bd}	2.50±0.32^{bc}	2.16±0.28^{be}	1.86±0.26^{be}	1.46±0.32^{be}	1.16±0.29^{be}
		(32.31)	(35.90)	(48.57)	(58.67)	(67.56)	(74.56)
1400	0.00±0.00	3.26±0.21^{adf}	2.94±0.12^{acf}	3.00±0.12^{afh}	2.64±0.11^{afh}	2.52±0.19^{afh}	2.06±0.32^{afh}
		(16.41)	(24.62)	(28.57)	(41.33)	(44.00)	(54.82)
Piroxicam 20	0.00±0.00	2.84±0.22^{cdf}	3.24±0.12^{adf}	2.34±0.35^{cei}	2.04±0.25^{cei}	1.74±0.18^{cei}	1.54±0.05^{ceh}
		(27.18)	(16.92)	(44.23)	(54.67)	(61.33)	(66.23)
N. Saline 0.2ml	0.00±0.00	3.90±0.09^{aef}	3.90±0.09^{aeg}	4.20±0.11^{dgj}	4.50±0.05^{dgj}	4.50±0.24^{dgj}	4.56±0.26^{dgi}

Discussion

The findings of this study reveal the presence of some important bioactive metabolite of therapeutic value namely alkaloids, cardiac glycosides, flavonoids, saponins, terpeniods and tannins. Different literatures supported the therapeutic importance of plants in the management of pain and inflammation. Amin et al., (2012) and Ahmadiani et al., (2000) reported the roles of flavoniods and tannins in the management of pain and inflammation. Similar activities were also reported by Reanmongkol et al., (2005) for alkaloidal and Choi et al., (2005); Arrau et al.,(2010) for saponins. Likewise, Fan et al (2004) attributed the anti-inflammatory activity of Terminalia catappa to terpenoids. In a recent publication, Shehu et al (2016) attributed analgesic effect to Saponin and Flavonoids using Murine models of pain. Thus, the anti-inflammatory and analgesic activity of the n-butanol partitioned portion of the aerial parts of Laggera aurita may also be due to the presences of these secondary metabolites which has the ability to inhibit phosphodiesterases involved in cell activation and mediate adhesion of circulating leucocytes at inflammatory sites (Duke, 1992, Abramson and Melton, 2000).

The mean pain response and percentage inhibition of the n-butanol portion in Table 2 revealed that, the most significant ($p<0.01$) inhibition was observed at 700 mg/kg in both phases, this inhibitory responses of the n-butanol portion of the extract exceed those observed with the crude in an earlier publication. This may in part be attributed to the alkaloids, terpenoids, flavonoids and saponins as reported above. Although, it is not easy to pin-point the exact phytoconstituent(s) responsible for the analgesic activity. Consequently, Ramadan et al. (1994) equally studied the anti-inflammatory and analgesic effects of Adansonia digitata and reported that the anti-inflammatory effect may be due to the presence of flavonoids, sterols, saponins and triterpeniods in the n-butanol portion, some of which are present in the plant under study. The two distinct phases observed after the injection formalin in the right hind paw of the rats are named the neurogenic phase (likely to be due to the direct effect on peripheral nociceptors activating primary afferent fiber), Substance P, glutamate and bradykinin are probably participating in this phase, which is thought to be non-inflammatory pain (Hunsker and Hole 1987). While the late phase was believed to be due to the release of inflammatory mediators, such as histamine and prostaglandin (Tjølsen et al 1992: Abbot et al 1995).

In the same vein, Table 3 revealed that, the n-butanol portion of the extract most significantly ($p<0.01$) inhibited the oedema more than the control at 700 mg/kg. Thus, the significant anti-inflammatory effect of the n-butanol portion may be due to the presence of flavonoids, saponins and alkaloids. This is supported by the facts that different types of saponins isolated from plants like Phytolocca americana, Madhuca longifolia and Carissa edulis have reportedly exhibited significant analgesic and anti-inflammatory activity (Singh et al., 1992, Hassan et al., 2010). Similarly, other types of saponins extracted from Hedera helix and Hedera colchica exerted anti-inflammatory activity by blocking bradykinin (Sparg et al., 2004) and other anti-inflammatory mediators such as the prostaglandins (Gepdiremen et al., 2005), likewise, alkaloids (Reanmongkol et al., 2005). Thus, the extracts might have acted via blockade of both bradykinin (a chemical peptide produce in the blood when tissues are injured) and prostaglandins (are unsaturated fatty acid found in mammals that performs function similar to hormones in controlling inflammation) to elicit their effect.

Conclusion

In conclusion, the n-butanol partitioned portion of the aeriel part Laggera aurita Linn. possessed significant anti-inflammatory and analgesic effects hence, supported the traditional use of the plant as drug in the management of pain and inflammation.

Acknowledgement

We will like to acknowledged the assistance of Mr. Fine Akawo of Chemistry Research Laboratory University of Maiduguri and Mr. Jostus Jibril of University of Maiduguri Teaching Hospital Maiduguri, Borno state Nigeria for their technical support.

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Cite this article:

Author(s), [1]Ali M. Fulata, [2]Hamidu Usman, [3]Arastus William, [2]Sule Ibrahim, [2]Adamu I. Zadva [2]Mohammed Babakura, [2]Halima A. Umar (2019). "Evaluation of Phytochemical Constituents, Analgesic and Anti-Inflammatory Efficacy of the N-Butanol Partitioned Portion of the Aerial Parts of Laggera Aurita Linn. In Albino Rats.", Name of the Journal: International Journal of Academic Research in Business, Arts and Science, (IJARBAS.COM), N, 295-305. DOI: 10.5281/zenodo.3369849, Issue: 2, Vol.: 1, Article: 16, Month: August, Year: 2019. Retrieved from https://www.ijarbas.com/all-issues/current-articles/

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