Chemistry Research Journal, 2017, 2(5):22-28

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Phytochemical, Analgesic and Anti-inflammatory Evaluation of the Crude Ethanol and *n*-hexane Extracts of the Aerial Parts of *Laggera aurita* Linn.

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Abstract The use of herbs to treat disease is almost universal among non-industrialized societies, and is often more available, accessible and affordable than purchasing expensive modern pharmaceuticals. *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. This paper seeks to examine the 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 and as well as to evaluate its phytoconstituents. The results of the phytochemical evaluation of the extracts of the aerial part of *Laggera aurita* in revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, terpenoids and tannins. The results of the pharmacological studies carried out revealed that, the crude extract showed a significant protection against inflammation and pain while the *n*-hexane defatted portion of the aerial part of *laggera aurita* posses lower activity against inflammatory and pain.

Keywords Analgesic, Anti-inflammatory, Phytochemistry, *Laggera aurita*, ethanol, *n*-hexane

Introduction

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that perform important biological functions, including defense against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 of such compounds have been isolated so far; a number estimated to be less than 10% of the total [1]. Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be comparatively as effective as conventional medicines, but also gives them the same potential to cause harmful side effects [2].

The genus *Laggera* is found in the Tropical and Sub-Tropical zones of Asia, especially the Indian Subcontinent and Southeast Asia. A few species are found in Australia and still fewer in Africa. The plants of this genus are mostly small weeds. Some of them are ruderal species. A few of the species were formerly included in genus *Conyza*. Many species of genus *Laggera* are used in traditional Chinese medicine. There are several literatures supportive of the

therapeutic effects of *L. aurita* among African countries among which are Benin republic, Ghana, Cameroon Sudan and Nigeria with traditional application in the treatment and management of connective tissues, inflammatory conditions, pain, fever and jaundice, as well as rheumatism and showed diuretic properties among pregnant women [3-5].

This paper seeks to examine the phytoconstituents, analgesic and anti inflammatory effects of the ethanolic extract and the *n*-hexane defatted portion of the aerial part of *Laggera aurita* Linn. using formalin induced pain and egg albumin induced inflammation in rats.

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*.

Sample Collection, Sample 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 resulting mass were then weighed and subjected to phytochemical and pharmacological evaluations using standard procedures.

Phytochemical Evaluation of the aerial part of *Laggera aurita*

Phytochemical evaluations were carried out on the ethanolic crude extract and the n-hexane defatted portion of the aerial part of *Laggera aurita* as using standard procedures as reported by; [14; 15;16; 17; 18; 19] for alkaloids, anthraquinones, flavonoids, tannins, saponins and phlobatannins.

Anti-inflammatory Test

Five (5) groups of five (5) rats were administered each of the two extracts of the aerial part of *Laggera aurita* (400, 800 or 1600 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 [20], 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% [21].

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

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

Where: vc = control represent the groups administered normal saline (negative control) and vt = treatment represents the groups administered extracts and piroxicam [22].

Analgesic Test

Twenty five (25) adult rats of both sexes were grouped randomly into five (5) groups of five (5) rats for each extract. 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: 400, 800 and 1600 mg/kg bd.wt of the two extracts respectively. All the treatments were done *intraperitoneally*. Thirty (30) minutes after administration of the extract, 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 [23]. The rats were observed for the first 5 minutes and then from 20-30 minutes after formalin injection.

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

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

Where: vc = control represent the groups administered normal saline (negative control)
vt = treatment represents the groups administered extracts and piroxicam [22].

Results

Tabla1 shows the results of the phytochemical evaluation of the crude ethanol extract and n-hexane portion of the aerial part *Laggera aurita* Linn.

Table 1: Phytochemical Constituents of the Ethanol Extract of the Aerial Part of *Laggera aurita*

Phytochemical Evaluation	Ethanol extract	*n*-hexane Portion
Phlobatannins	-	-
Anthraquinones	-	-
Carbohydrates	+	-
Flavonoids	+	+
Alkaloids	+	+
Terpenoids	+	-
Tannins	+	-
Saponins	+	-
Cardiac glycosides	+	-

Key: (+) Present, (–) Not detected

Table 2: Results of the egg albumin induced inflammation in rats of the crude ethanol extract of the aerial part *Laggera aurita* Linn.

Treatment/	Time (min.)/ Oedema Level (Mean ± SEM) mm						
Dose (mg/kg)	0	20	40	60	80	100	120
400	0.00±0.00	3.52±0.24^{a} (9.74)	3.36±0.17^{a} (13.85)	2.82±0.31^{a} (32.86)	2.42±0.29^{a} (46.22)	2.12±0.37^{a} (52.89)	1.72±0.26^{a} (62.28)
800	0.00±0.00	4.48±0.21^{be} (14.87)	3.18±0.37^{ab} (18.46)	3.38±0.25^{ac} (19.52)	3.02±0.21^{be} (32.89)	2.58±0.19^{ac} (42.67)	2.18±0.14^{ac} (52.19)
1600	0.00±0.00	2.78±0.15^{cfi} (28.72)	2.58±0.16^{abc} (33.85)	2.38±0.25^{ace} (43.33)	2.10±0.11^{afi} (53.33)	1.74±0.09^{ace} (61.33)	1.48±0.16^{ace} (67.54)
Piroxicam 20	0.00±0.00	2.84±0.22^{dgi} (27.18)	3.24±0.12^{abc} (16.92)	2.34±0.35^{ace} (44.23)	2.04±0.25^{cgi} (54.67)	1.74±0.18^{ace} (61.33)	1.54±0.05^{ace} (66.23)
N. Saline	0.00±0.00	3.90±0.09^{ahj}	3.90±0.09^{abd}	4.20±0.11^{bdf}	4.50±0.05^{dhj}	4.50±0.24^{bdf}	4.56±0.26^{bdf}

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

Table 3: Results of the egg albumin induced inflammation in rats of the *n*-hexane defatted portion of the aerial part *Laggera aurita* Linn.

Treatment/	Time (min.)/ Oedema Level (Mean ± SEM) mm						
Dose (mg/kg)	0	20	40	60	80	100	120
350	0.00±0.00	2.98±0.32^{a} (23.59)	2.82±0.16^{a} (27.69)	2.98±0.30^{a} (29.05)	2.32±0.17^{a} (48.44)	2.26±0.22^{a} (49.78)	1.60±0.31^{a} (64.91)
700	0.00±0.00	3.44±0.25^{ac} (11.79)	3.24±0.22^{ad} (16.92)	3.16±0.25^{ac} (24.76)	3.14±0.27^{be} (30.22)	3.00±0.29^{ac} (33.33)	2.80±0.23^{be} (38.60)
1400	0.00±0.00	3.68±0.21^{acd} (5.64)	4.00±0.23^{beg} (2.56)	3.54±0.22^{ace} (15.71)	3.64±0.43^{ceh} (19.11)	3.08±0.20^{acf} (31.56)	3.14±0.43^{ceh} (31.14)
Piroxicam 20	0.00±0.00	2.84±0.22^{acd} (27.18)	3.24±0.12^{adh} (16.92)	2.34±0.35^{acf} (44.23)	2.04±0.25^{afi} (54.67)	1.74±0.18^{adg} (61.33)	1.54±0.05^{afi} (66.23)
N. Saline	0.00±0.00	3.90±0.09^{bcd}	3.90±0.09^{cfg}	4.20±0.11^{bde}	4.50±0.05^{dgj}	4.50±0.24^{beh}	4.56±0.26^{dgj}

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

Table 4: Results of the formalin induced pain in rats of the crude ethanol extract of the aerial part *Laggera aurita* Linn.

Treatment/Dose	No. of Paw Licking (Mean ± SEM)			
(mg/kg bd.wt)	0 -5 min.	(%)	20-30min.	(%)
400	48.00±7.76[a]	41.75	46.80±3.43[a]	68.92
800	43.80±7.03[ad]	47.84	34.4±3.93[ac]	77.16
1600	48.60±3.83[adf]	41.02	48.4±7.94[ace]	67.86
Piroxicam 20	23.80±6.31[bdg]	71.12	38.20±7.85[ace]	74.63
Normal saline 0.2 ml	82.4±4.29[ceh]		150.60±11.35[bdf]	

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

Table 5**:** Results of the formalin induced pain in rats of the *n*-hexana portion of the aerial part *Laggera aurita* Linn.

Treatment/Dose	No. of Paw Licking (Mean ± SEM)			
(mg/kg bd.wt)	0 -5 min.	(%)	20-30min.	(%)
350	48.00±7.76[a]	15.05	46.80±3.43[a]	**64.94**
700	43.80±7.03[ad]	19.17	34.4±3.93[ac]	**59.10**
1400	48.60±3.83[adf]	39.56	48.4±7.94[ace]	**50.33**
Piroxicam 20	23.80±6.31[bdg]	71.12	38.20±7.85[ace]	**74.63**
Normal saline	82.4±4.29[ceh]		150.60±11.35[bdf]	

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

Discussion

The results of the phytochemical evaluation of the aerial part of *Laggera aurita* in Table 1 revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, terpenoids and tannins, while, the results of the *n*-hexane defatted portion revealed the presence of terpenoids and alkaloids, which are reported to possessed several pharmacological activities among which are anti-inflammatory and analgesic. Analgesic and anti-inflammatory effects have been observed and reported with flavonoids [24] and tannins [25]. There are also reports on alkaloidal analgesic effects [26], as well as saponins [27; 28; 29] which are attributed to the anti-inflammatory activity of *Terminalia catappa* to terpenoids. Several flavonoids isolated from medicinal plants have been shown to possess significant anti-inflammatory activity [30; 24]. For instance, an isolated flavonoid fraction from *Celosia argentea Linn* showed significant dose dependent anti-inflammatory activity [6]. This was supported by other workers who found that flavonoids inhibit phosphodiesterases which are involved in cell activation. The effect is dependent on biosynthesis of protein cytokines that mediate adhesion of circulating leucocytes to the sites of injuries [31; 32]. Thus the anti-inflammatory activity of the aerial parts of *Laggera aurita* may also be due to its ability to inhibit phosphodiesterases involved in cell activation and mediate adhesion of circulating leucocytes at inflammatory sites.

The anti-inflammatory effect and the percentage protection of the crude extract of the aerial parts of *Laggera aurita* on egg albumin induced oedema in rats in Table 2 showed that there is no significant difference along the column among the treatment group. The crude ethanolic extract inhibited the oedema in the early phase as well as in the late phase, the extract oedema inhibitions was most significant ($p<0.01$) at 800 mg/kg bd.wt. The crude extract acted dose dependently in both phases but the rats were observed to be sedated at the highest dose. Similarly, the mean oedema level and the percentage inhibition of the n-hexane defatted portion of the aerial parts of *L. aurita* extract in Table 3 revealed that the extract at the lowest dose (350 mg/kg bd.wt) significantly ($p<0.05$) inhibited the oedema in the early phase, but the inhibition slightly decreases with increase in the dose. 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. However, the effects of the extracts were more pronounced in the late phase in both models. The effects manifested in the late phase may be due to inflammation causing a release of serotonin, histamine, bradykinin and prostaglandins, which at least to some degree can cause the sensitization of the central nociceptive neurons [33]. Drugs that inhibit the first phase of the formalin-induced nociception test have the

ability to alleviate neurogenic pain while those drugs that inhibit the second phase of the test have the ability to inhibit inflammatory pain [34]. In living animal tissues, inflammatory processes involve the release of several mediators which include prostaglandins, histamine and cytokines and substances that regulate adhesion of molecules and cell migration, activation and degeneration [35; 36].

More so, the analgesic effect of the ethanol crude extract of the aerial part of *Laggera aurita* on formalin-induced pain in rats and the percentage inhibition in Table 4 showed that the extract significantly inhibited the pain response at the different dosages with the most significant inhibition observed at 800 mg/kg in both phases. The standard drug piroxicam had also significantly ($p<0.05$) showed a great analgesic activity by blocking both phases of the formalin-induced nociception. likewise, the mean protection and the percentage inhibition of the n-hexane defatted portion in Table 5 revealed that the n-hexane defatted portion of the extract slightly inhibited the pain response in dose dependant manner in the early phases, while in the late phase 350 mg/kg shows the better activity than two higher doses (700 and 1400 mg/kg), but however, all the treatment groups were significantly lower than the mean protection and percentage inhibition of the positive control (Piroxicam).

Hence, based on the results presented, the findings of this study corroborates with the findings of many scholars who had worked on different plants using different models, among which were; the alcoholic extract of *Celosia argentea* [6], the aqueous leaves extract of *Ocimum gratissimum* shows a significant dose dependent antinociceptive effect via acetic acid induced writhing and hot plate method, and also anti-inflammatory activity [7]. So also, Gossypin, an isolated bioflavonoid from the yellow petals of *Hibiscus vitofolius* has been shown to possess anti-nociceptive activity similar to morphine and has the advantage of lack of tolerance and dependence liability [8]. The n-butanol soluble fractions of the methanol leaf extract of *Cissus cornifolia* showed analgesic and anti-inflammatory activity via acetic acid induced writhing, hot plate method and carrageenan induced inflammation [9]. The flavonoid-rich fraction from *Celosia argentea* possesses significant anti-inflammatory effect when evaluated using carrageenan induced inflammation and cotton pellet induced granulomatosus models [10]. Other African medicinal plants with anti-inflammatory activities include *Khaya senegalensis* [11], *Acanthus montanus* [12] and *Desmodium gangeticum* [13].

Conclusion

In conclusion, the based on the experiments carried out the crude extract showed a significant protection against inflammation and pain while the *n*-hexane defatted portion of the aerial part of *Laggera aurita* posses lower activity against inflammatory and pain. Therefore, further studies should be carried out using different models and route of administration to ascertain exact cause of the lower effects observed with the *n*-hexane portion in this paper.

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