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Phytochemical Screening, Proximate Analysis and Acute Toxicity Study of *Launaea Taraxacifolia* Ethanolic Extract on Albino Rats

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Abstract:
Despite rapid growth in the use of herbal medicines, there are inadequate research evidences on their effectiveness and toxicity. Launaea taraxacifolia is a very important neutraceutical herb in the West African Sub-region. Phytochemicals and proximate contents showed that this vegetable might have medicinal value in man. This study therefore investigated its safety in rats. The acute toxicity studies were carried out based on OECD guidelines 423 and fixed dosage studies were adopted where the limit dose was 3000mg/kg body weight of test animal. A total number of fifteen (15) albino rats were grouped into 5 study groups of 3 rats each. Group: I (control), II (500mg/kg), III (1000mg/kg), IV (2000mg/kg) and V (3000mg/kg). Acute toxicity studies of the extracts on rats did not show any form of death when examined for 7 days. The highest dose administered (3000mg/kg body weight) did not produce mortality or changes in the general behavior of the test animals. The histopathological studies of the liver and the kidney tissues unveiled some structural changes in the organs of rats treated with different concentrations of the methanolic extracts of the herbs. This may have potentiated the toxic effect. Therefore, indiscriminate use must be avoided. The body and organ weight (liver and kidney) were not affected by the administered extracts.

Keywords: *Toxicity, launaea taraxacifolia, ethanolic extract, phytochemicals, proximate analysis*

1. Introduction

Launaea taraxacifolia occurs mainly in the tropic regions of Senegal, Ethiopia and Tanzania. The Ethiopian highlands have been suggested as the place of origin, from where it was introduced elsewhere and spread as a weed. *Launaea taraxacifolia* has been domesticated as a leafy vegetable in Nigeria, and is also cultivated locally in Senegal and Benin (Adebisi, 2004). It is known as 'yanrin' among the Yorubas of the South-Western part of Nigeria, 'ugu' among the Ibos of the Eastern part of Nigeria and 'nonon barya' among the Hausas of the Northern part of Nigeria. It is an erect perennial herb with leaves at the base of the stem in a rosette form. The higher leaves are articulate and toothed with the lower leaves tapering at the base. Apart from its being used as a common vegetable, it is also eaten by some people as salad, cooked in soups and sauces. *L. taraxacifolia* leaves are fed to lactating cows in northern part of Nigeria to increase their milk production; it is also used to enhance multiple birth rates in sheep and goats (Burkill, 1985). In recent times, plants research has received devoted interest in Western world and effort channeled into the discovery of new biologically active molecules and crude extracts of plants for self medication by the general public (Peter, 1995). Medicinally, the leaves are rubbed on limbs to make children in Nigeria and Ghana walk, their leaves are also mixed with ashes to cure yaws (Ayensu, 1978). A previous study by Okafor in 1983 reported that vegetables are not only cheap sources of nutrients but are also common sources. Fruits and vegetables are the greatest sources of phytochemicals and facts have emerged that some ant nutritional content of these vegetables has potentials in reducing some diseases in man (Alta and Adeogun, 1995; Williamson et al*.,* 1997). Some of these diseases include high blood pressure, heart attack, stroke and other cardiovascular diseases (Williamson et al., 1997). The phytochemicals studied showed their healing activities by combining with vitamins and other nutrients. *Lactuca taraxacifolia* of the family Asteraceae was formally called *Launaea taraxacifolia* which is not very common compared with *S. aethiopicum* and *T. triangulare*. It has been observed to be a good source of phosphorous, iron and protein. The best time of harvest appear to be between 6th and 8th week after planting (Sakpereand and Aremu, 2008). It has medicinal properties; consumption of *L. taraxacifolia* could

prevent infection and further replication of measles as reported by Obi (et al., 2006). In animals, evaluation has shown that it has anti-hypertensive effect (Adebisi, 2004).

A number of studies have reported the toxic effects of herbal medicines (Gamble, 1957; Calixto, 2000; Jaound et al., 2004; Taziebou et al., 2008). Studies on medicinal plants showed that various phytochemicals in medicinal plants exhibit ameliorative effect on many ailments. *L. taraxacifolia* is one of the traditional leafy vegetables in Nigeria which is currently endangered, threatened with extinction and is grossly understudied with no records of production (Adebisi, 2004). Adeboye et al (2003) reported that most of the leafy vegetables are not easily available as farmers now gather them with great drudgery and difficulty from the few available stands.

2. Materials and Methods

2.1. Plant Collection and Extraction

Fresh plant leaves were collected from the Obafemi Awolowo University, Ile-Ife farm and authenticated by Botany Department, Joseph Ayo Babalola University, Ikeji Arakeji, Osun State. The fresh plant material was then washed under running tap water, air dried, homogenized into fine powder and stored in air-tight containers at 4°C. About 100g of pulverized air dried leaves of *L. taraxacifolia*, was mixed with 500ml of 70% ethanol in a conical flask, plugged with cotton wool and then kept on a shaker for 72 hours. The mixture was then filtered and the solvent was evaporated using rotary vacuum pump. The crude extract obtained was stored in an air-tight desiccator for further analysis.

2.2. Phytochemical Screening and Proximate Analysis

Phytochemical test on the leaves of *L. taraxacifolia* was carried out on the crude ethanolic extracts using standard procedures (AOAC, 1984). The estimation of food parameters namely; moisture content, total ash, crude fat, crude fibre, crude protein and total carbohydrate on dry matter were determined according to standard procedures using 2g of dried powdered sample. In crude protein determination, Nitrogen was determined by Kjeldahl method (Pearson, 1976) and converted to protein by multiplying by a factor of 6.25. Moisture content, crude fat, crude fibre and total ash were determined by AOAC (1984) and total carbohydrate was determined using James method (1995) computed using the equation:

Total carbohydrate = 100-[%crude protein+ %crude fat+ %crude fibre + %crude total ash]

Determination of energy or calorific value: The total energy value in the leaves of *L. taraxacifolia* in kcal/100 g was estimated using the method described by FAO (2003) as shown below:

Energy value=[% crude proteinx4.0]+[% crude fat x 9.0]+[%Carbohydrate x 4.0]

Experimental protocol and animal treatment

15 albino wistar rats were purchased from animal house Biochemistry department, University of Ibadan. The animals were housed in polypropylene cages (55 x 32.7 x 19cm), with sawdust litter in a temperature controlled environment (23 ± 2ºC). Lighting was controlled to supply 12 hours of light and 12hours of dark for each 24hours period. Each cage was identified by a card. This card stated the cage number, number and weight of the animals it contained, test substance code, administration route and dose level. The animals were fed with standard laboratory animal food pellets and water for two weeks of acclimatization in the animal house department of Chemical Sciences, Joseph Ayo Babalola University Osun State-Nigeria. The animals of both sexes weighing between 55-130g, nulliparous and non-pregnant were randomly divided into five study groups labeled I, II, III, IV and V with each group containing three rats for the ethanolic extracts study. Acute oral toxicity test was performed using Organization for Economic Co-operation and Development (OECD) guidelines 423 of 2011.

The test substance was administered in a single dose by gavage using a locally designed rat oral needle for acute toxicity study. Experimental Animals were starved for 3 hours of food pellets prior to dosing. Consequent upon fasting, animals were weighed and test substance was administered orally at a dose of 0 mg/kg, 500mg/kg, 1000 mg/kg, 2000 mg/kg and 3000 mg/kg respectively. After the administration of test substance, foods for the rats were withheld for 4 hours. The extract was prepared by dissolving 500mg-3000mg of dried powder of *L. taraxacifolia* leaves in 1ml of distilled water and a dose of 1ml/kg body weight was administered. The volume of extract to be administered was determined based on body weight and given to the rat once. The toxicological effects were observed in terms of mortality expressed as LD_{50}. At the end of the experiment, rats were sacrificed liver and kidney were dissected, weighed and directly fixed in 10% formalin prior to processing. It was later washed thoroughly in normal saline, trimmed, processed, embedded in paraffin and sectionalized at a thickness of 4-5µm. Mayer's Acid-Alum-Haematoxylin and eosin was adopted (Beaker *et al.,* 1998) and photomicrograph taken accordingly.

2.3. Statistical Analysis

The SPSS (Statistical Package for Social Sciences) software packages version 16 were used for statistical analysis. Results were presented as mean and standard error (Mean ± S.E). The statistical significance between the control and each of the treated groups was determined by Dennett's t- test after one-way ANOVA. The level of significance was set at $P < 0.05$.

3. Results

Phytochemical constituents	Observation	Inference
Alkaloid	No yellow precipitate formed	-
Flavonoids	Yellow colour persist	+
Saponins	Yellow emulsion formed	+
Terpenoids	Reddish brown colour	+
Steroids	Reddish brown colour	+
Cardiac glycosides	Yellow brown ring of upper layer	+
Tannins	Green black colour	+
Anthraquinones	Green colour	-

Table 1: Phytochemical screening of ethanolic extract of Launaea taraxacifolia
+: present, -: absent. Assays were all carried out in triplicates

Parameter	Percentage Value (%)
Total Carbohydrate	18.59 ±1.33
Crude Protein	17.67±120
Crude Ash	21.50±0.07
Crude Fibre	16.06±0.05
Crude Fat	4.70±0.03
Moisture Content	23.14±0.50
Calorific Value (Kcal/100g)	280.70±0.80

Table 2: Proximate composition of Launaea taraxacifolia on dry matter basis
Ash Value expressed as % mean ± SEM, n=3

S/N	Response	Group observation				
		I (0mg/kg)	II (500mg/kg)	III (1000mg/kg)	IV (2000mg/kg)	V (3000mg/kg)
1.	Alertness	Yes	Yes	Yes	No	No
2.	Grooming	Normal	Normal	Normal	Normal	Normal
3.	Touch response	Yes	No	No	No	No
4.	Torch response	Yes	Yes	Yes	Yes	Yes
5.	Tremor	No	No	No	No	No
6.	Convulsion	No	No	No	No	No
7.	Gripping strength	Normal	Reduced	Reduced	Reduced	Reduced
8.	Response to food	Yes	No	No	No	No
9.	Pupils	Normal	Normal	Normal	Normal	Normal
10.	Urination	Normal	Normal	Normal	Normal	Normal
11.	Salivation	No	No	No	No	No
12.	Hyperactivity	Normal	Reduced	Reduced	Reduced	Reduced
13.	Skin colour	Normal	Normal	Normal	Normal	Normal
14.	Corneal reflux	Normal	Normal	Normal	Normal	Normal
15.	Pinna reflux	Normal	Normal	Normal	Normal	Normal
16	Sound response	Normal	Normal	Normal	Normal	Normal

Table 3: Effect of ethanol extracts of Launaea taraxacifolia on acute oral toxicity in rats during Observation in first 6 hours
Source: Lalitha et al., 2012. Asian Journal of Pharmaceutical and Clinical Research

Group	Conc. of plant extract (mg/kg)	Body weight(g)		Difference in weight (g)	Liver weight(g)	Kidney weight(g)
		Final	Initial			
I	0	95.10±26.45	94.26±26.16	0.84±0.28	3.67±0.86	0.50±0.15
II	500	78.60±16.69	78.56±18.10	0.04±1.41	3.27±0.25	0.61±0.19
	t-,p-value	0.75,0.550	0.70, 0.560	0.78, 0.515	0.64, 0.587	-0.58,0.618
III	1000	82.95±14.92	77.56±8.90	5.39±6.01	4.10±0.14	0.67±0.04
	t-,p-value	0.56,0.641	0.85, 0.527	-1.06,0.478	-0.69,0.560	-1.51,0.270
IV	2000	64.65±3.04	54.76±12.45	9.89±9.40	4.04±0.06	0.60±0.14
	t-,p-value	1.61, 0.348	1.92, 0.242	-1.36,0.403	-0.61,0.604	-0.65,0.580
V	3000	71.67±3.86	70.16±4.53	1.51±0.66	3.95±0.07	0.67±0.04
	t-,p-value	1.24, 0.341	1.23, 0.328	-1.31,0.320	-0.45,0.695	-1.51,0.270

Table 4: Body weight indices after the administration of varied concentration of ethanolic extracts of Lausnaea taraxacifolia leaves to rats and organ weight after sacrifice
The results are the means of 3 determinations ± S.D P-value significant at < 0.05

4. Discussion

The present study conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines 423 of 2011 revealed that the said extracts did not produce any mortality throughout the study period of 7 days even when the limit dose of 3000mg/kg body weight was maintained. The oral LD_{50} was indeterminable being in excess of 3000mg/kg body weight. So, testing the extracts at a higher dose may be necessary and the extracts were practically non-toxic. Phytochemical screening of the ethanolic extract of the leaf of *Launaea taraxacifolia* showed the presence of flavonoids, saponins, terpenoids, steroids, cardiac glycosides and tannin. Results obtained for proximate analysis of *Launaea taraxacifolia* ethanolic extract of the leaf showed high calorific value, total carbohydrate, crude protein, crude fibre, and total ash while crude fat is the lowest. Acute toxicity showed that the LD_{50} was above 3000mg/kg in the treated rats. The administered graded doses of the ethanolic extract did not result in lethality over the 24 hour period. No death and latent toxicity was observed in the animals after keeping them for extra 7 days. Body weight indices after the administration of varied concentration of ethanolic extracts of *Launaea taraxacifolia* leaves to rats and comparison of organ weight to the control after sacrifice increased significantly.

5. Conclusion

Phytochemical screening of the ethanolic extract of the leaf of *Launaea taraxacifolia* showed the presence of flavonoids, saponins, terpenoids, steroids, cardiac glycosides and tannin.

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