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Comparative anti-anaemic activity of methanolic extracts of *Momordica charantia* and *Luffa acutangula*

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Abstract

The present research work mainly focused on the preparation of methanolic extracts of *Momordica charantia* and *Luffa acutangula* fruits and their pharmacological evaluation for anti-anaemic property individually. The methanolic extracts were prepared by simple maceration process. Preliminary phytochemical screening of the extracts showed the presence of various phytochemicals. Anti-anaemic activity was evaluated on wistar rats using phenyl hydrazine induced anaemia model. The results of extract treated groups were compared with that of the anaemic group. Dexorange syrup was used as standard. Blood samples were collected through retro-orbital puncture from rats on day 0, 2, 7, 14, 21 and 28 of treatment and subjected to analysis of red blood cell count, haemoglobin and hematocrit using 3-part haematology analyzer. A significant increase in red blood cell count, haemoglobin and hematocrit was observed in rats treated with methanolic extract of *Momordica charantia* and *Luffa acutangula* when compared to that of the untreated anaemic group. *Momordica charantia* showed more anti-anaemic effect when compared to *Luffa acutangula*. Thus from the present study, it was concluded that the *Momordica charantia* and *Luffa acutangula* showed significant anti-anaemic activity.

Keywords: Anaemia; haemoglobin; phenyl hydrazine; rats.

Introduction

Anaemia is a condition where there is a decrease in the total amount of red blood cells (RBCs) and hemoglobin in the blood. It is affecting about a quarter of people globally. It is more common in females, than in males(1). Haemolytic anaemia occurs due to abnormal breakdown of RBC, either in blood vessels or elsewhere in the human body. It causes several consequences, ranging from relatively harmless to life-threatening (2).Haemolytic anaemia may be due to oxidative stress or defects in RBC membrane production and haemoglobin production. It shows symptoms like fatigue, pallor, loss of weight, shortness of breath and dark colored urine. Anaemia can be induced by several chemicals. One of them is phenyl hydrazine. It causes haemolytic anaemia by increasing the absorption of iron in liver, spleen and duodenum and there by leading to altered iron metabolism in body.

Plants have been used for health and medicinal purposes for several thousands of years. They support in relieving from several types of ailments (3). *Momordica charantia* and *Luffa acutangula* belonging to the family *Cucurbitaceae* have several therapeutic benefits. *Momordica charantia*, commonly called as “Bitter gourd” and *Luffa acutangula*, commonly called as “ridged gourd” are available throughout India and are extensively used as vegetables. The fruits of *Momordica* have a distinct warty exterior and an oblong shape. The fruit is most often consumed green. It is mostly used in culinary. These are useful in treatment of diabetes mellitus, anthelmintic, anaemia and rheumatoid arthritis (4). The fruits of *Luffa* are slightly bitter in taste with slightly spongy texture. These are useful in killing of parasites and to treat gonorrhea, eczema and anemia (5). The present study was aimed on the preparation of methanolic extracts from unripe fruits of *Momordica charantia* and *Luffa*

Vol. 16 (Supplementy Issue 2) 155 - 161, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.73

acutangula and to evaluate their anti-anaemic potential on Wistar rats.

Materials and Methods

***Plant Material*:** Fruits of *Momordica charantia* and *Luffa acutangula* belonging to the family *Cucurbitaceae* were collected from the local market of Guntur, Andhra Pradesh, India. Collected material was analyzed and authentified by Dr. K. Ammani, Professor, Department of Botany, Acharya Nagarjuna University. A voucher specimen (02/2017 and 03/2017) was preserved in the Department of Pharmacology.

Preparation of Methanolic Extract of Momordica charantia and Luffa acutangula: The fruits of *Momordica charantia* were made into small pieces, dried, powdered and subjected to maceration. In this a total amount of 100 g powder is macerated in 400ml of methanol for 72 hours with occasional stirring for every 3 hours. At the end, the extract was passed through a filter paper and filtrate was evaporated on water bath to obtain crude. After cooling 2 drops of chloroform are added for preservation. Condensed extracts were weighed and stored in air-tight containers at 4^0C till further investigation. Similar procedure was followed to extract *Luffa acutangula* fruits.

Phytochemical Analysis: Preliminary phytochemical screening was performed for the methanolic extracts of *Momordica charantia* and *Luffa acutangula* to detect the presence of various constituents responsible for the pharmacological activity like carbohydrates, fixed oils, glycosides, alkaloids, flavonoids, tannins, polyphenols, steroids and saponins (6).

***Drugs and Chemicals*:** Dexorange syrup (Franco-Indian Pharmaceuticals Pvt. Ltd., Mumbai) and phenyl hydrazine hydrochloride (Qualigens Fine Chemicals, Mumbai) were commercially procured from. All other materials used were of analytic grade and procured commercially.

Experimental Animals: Healthy adult male albino rats (Wistar strain) weighing 250–300 g, housed in polypropylene cages, maintained under standardized condition i.e., 12:12 hour light/dark cycle at 25 ± 2^0C with paddy husk bedding at the animal house, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Guntur, India. Animals were provided with standard pellet food and had free access to purified drinking water.

Pharmacological Evaluation:

***Induction of Anaemia*:** Anaemia was induced in all groups of rats except to the control group, by intra-peritoneal administration of 40 mg/kg of phenyl hydrazine (PHZ) for two days (7).

***Treatment of Animals*:** Rats were divided into five groups and treated daily for 4 weeks. The control group I is treated with normal saline solution. The anaemic control group II was treated with phenyl hydrazine 40 mg/kg for first two days. The standard group III was treated with dexorange syrup at a dose of 1ml/day from day 2 to day 28. Whereas the test groups IV and V were treated with methanolic extract of *Momordica charantia* and *Luffa acutangula* respectively at a dose of 200 mg/kg from day 2 to day 28. This dose was selected based on the acute toxicity tests from past studies (8-9). All administrations were done orally using oropharyngeal cannula once per day for 28 days (4 weeks).

Analysis of Haematological Parameters: Blood samples were collected from the rats through retro-orbital puncture, before induction of anaemia (day 0), after induction of anaemia with phenyl hydrazine (day 2) and on 7, 14, 21 and 28 days of treatment. The red blood cell count, haemoglobin concentration and haematocrit were determined before starting of the study and on 2, 7, 14, 21 and 28 days using a 3-part haematology analyzer (BENESPHERA) and the variations of average values of haematological parameters were calculated relative to the mean values of D0 and D2 (10).

Vol. 16 (Supplementy Issue 2) 155 - 161, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.73

***Statistical Analysis*:** Graph Pad Prism 5.0 software was used for the analysis of the results obtained. The mean value is accompanied by the standard error of mean (mean ± SD).

Results and Discussion

The present study was intended to prepare methanolic extracts of *Momordica charantia* and *Luffa acutangula* and to evaluate their anti-anaemic property.

***Phytochemical Screening*:** The phytochemical investigation of methanolic extract of *Momordica charantia* revealed the presence of alkaloids, carbohydrates, tannins, flavonoids, glycosides and saponins which promises their huge pharmacological abilities. The methanolic extract of *Luffa* contains all the phytochemicals except glycosides (Table 1). Past studies indicate that the phytochemicals that are present in the methanolic extracts of these plants have antioxidant activity which favours tissue regeneration and enhances the resistance of blood vessels to haemolysis (11). Alkaloids also been known to posses anti-anaemic property by inhibiting phosphodiesterase enzyme thereby accumulating cyclic adenosine monophosphate (cAMP). This causes phosphorylation and synthesis of proteins, which increases erythropoiesis (12). Saponins are also known to inhibit platelet aggregation and thrombosis. The methanolic extracts can detoxify the saponins which allow increase in haemoglobin and RBC(13). Flavonoids have anti-anaemic potential and also protect the blood capillaries(14).

Table 1. Phytochemical Screening of Methanolic Extracts of *Momordica Charantia* and *Luffa Acutangula*

Phytochemical Constituents	Methanolic Extracts of	
	Momordica charantia	*Luffa acutangula*
Alkaloids	+	+
Carbohydrates	+	+
Steroids	–	+
Tannins	+	+
Flavonoids	+	+
Glycosides	+	–
Saponins	+	+
+ indicates Presence ; – indicates Absence		

In vivo Anti-Anaemic Activity

***Effect on Body Weight*:** The *in vivo* anti-anaemic activity was performed by using phenyl hydrazine hydrochloride induced anaemia method. After administration of phenyl hydrazine, there is a decrease in the body weights of rats from all groups except normal group. The loss of body weight is one of the symptoms of anaemia, which would be due to lack of appetite in anaemic rats. This is mostly due to improper carbohydrate metabolism. After the study duration (28 days), the methanolic extract of *Momordica charantia* showed 14.50% increase in body weights of rats which are nearer to the standard group (17.65%). This indicates regaining of appetite in rats during treatment which lead to gain in body weight (15). The same was also observed in the rats that were treated with dexorange syrup (Table 2).

***Effect on Red Blood Cell Count*:** The administration of phenyl hydrazine leads to decrease in red blood cell count except in normal group of rats (16). An increase in number of red blood cells was observed in following weeks in treatment groups. The animals were almost recovered by the end of the study (28 days). Significantly, the animals treated with methanolic extract of *Momordica charantia* showed 80.88% increase in red blood cell count. A bit less effect than this was found with *Luffa* extract (Table 3).

***Effect on Haemoglobin Content*:** Haemoglobin is an important constituent of blood which maintains RBC in constant functioning and fulfils body oxygen needs. Administration of phenyl hydrazine on D2 caused a significant decrease in haemoglobin content in all groups except normal group(17).

Vol. 16 (Supplementy Issue 2) 155 - 161, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.73

Table 2. Effect of Methanolic Extracts of *Momordica charantia* and *Luffa acutangula* on Body Weight in Wistar Rats

Group	Treatment	Body Weight (g); Mean ± SD (%)					
		Day 0	Day 2	Day 7	Day 14	Day 21	Day 28
I	Control	300±1.12	302±1.77	300±0.81	305±1.64	300±0.14	302±0.77
II	Anemic control	298±0.93	260±1.64 (-12.75)[a]	262±0.22	275±1.55	275±1.94	270±1.86 (+03.84)[b]
III	Dexorange syrup	299±1.24	255±1.32 (-14.71)[a]	262±1.20	282±3.02	290±1.62	300±1.54 (+17.65)[b]
IV	Methanolic extract of *Momordica*	300±1.02	262±1.07 (-12.66)[a]	275±1.88	282±2.87	290±1.77	300±1.99 (+14.50)[b]
V	Methanolic extract of *Luffa*	325±1.56	275±1.99 (-15.38)[a]	282±1.05	290±1.35	300±1.64	310±1.42 (+12.72)[b]
a - Percentage variation compared to day 0; b - Percentage variation compared to day 2							

Table 3. Effect of Methanolic Extracts of *Momordica charantia* and *Luffa acutangula* on Red Blood Cell Count

Group	Treatment	Red Blood Cells (10^6 cells/µL); Mean ± SD (%)					
		Day 0	Day 2	Day 7	Day 14	Day 21	Day 28
I	Normal Control	8.92±1.04	9.44±1.72	9.02±1.04	9.27±2.32	9.14±1.10	9.44±0.44
II	Anemic control	8.74±1.23	4.5±0.21 (-48.51)[a]	4.63±1.54	6.63±2.41	7.05±0.66	7.39±2.01 (+64.22)[b]
III	Dexorange syrup	8.05±2.41	4.21±1.24 (-47.70)[a]	6.20±0.53	6.93±1.72	7.28±0.89	7.93±1.64 (+88.36)[b]
IV	Methanolic extract of *Momordica*	6.62±2.55	4.29±1.25 (-35.19)[a]	6.72±1.70	7.05±1.62	7.25±2.81	7.76±1.36 (+80.88)[b]
V	Methanolic extract of *Luffa*	8.17±0.36	4.59±2.08 (-43.81)[a]	5.84±1.42	7.10±0.96	7.76±1.82	8.10±0.63 (+76.47)[b]
a - Percentage variation compared to day 0; b - Percentage variation compared to day 2							

After treatment, a progressive recovery was obtained. Animals treated with vitamin syrup showed higher recovery which is 80.41%. A nearer recovery value was obtained with Methanolic extract of *Momordica* which is 80%. Even the extract of *Luffa* also showed prominent effect (Table 4).

***Effect on Haematocrit*:** Haematocrit also called as packed cell volume (PCV) is the measurement of volume percentage of red blood cells in the blood. The administration of phenyl hydrazine also decreased the haematocrit levels. After four weeks, the haematocrit value observed was very high in case of animals treated with methanolic extract

Vol. 16 (Supplementy Issue 2) 155 - 161, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.73

of *Momordica* which is 90.51% when compared to day 2. This was more than the standard treatment group (Table 5). The intra peritoneal administration of 40mg/kg/day of phenyl hydrazine for two days in Wistar rats caused a decrease in the concentration of body weight, haemoglobin, red blood cells and haematocrit. The results obtained were similar to those of previous studies who observed a decrease of number of blood cells and haematocrit with a phenyl hydrazine administration(18-19). Considering the results of the groups IV, V and VI, the methanolic extract of *Momordica charantia* fruits have higher anti-anaemic potential than that of other vegetable extracts. The anti-anaemic effect of

Table 4. Effect of Methanolic Extracts of *Momordica charantia* and *Luffa acutangula* on Haemoglobin Content

Group	Treatment	Haemoglobin (in g/dL); Mean ± SD (%)					
		Day 0	Day 2	Day 7	Day 14	Day 21	Day 28
I	Normal Control	17.21±1.30	17.31±1.68	16.71±1.60	16.91±1.20	16.30±1.60	17.10±0.53
II	Anemic control	16.12±1.52	10.73±1.58 (-33.54)[a]	11.90±1.13	12.72±2.41	13.84±1.95	13.27±1.58 (+23.36)[b]
III	Dexorange syrup	15.70±2.03	9.77±1.72 (-38.21)[a]	12.33±0.74	15.10±1.92	17.22±1.58	17.56±0.95 (+80.41)[b]
IV	Methanolic extract of *Momordica*	14.31±1.63	9.08±0.31 (-37.06)[a]	11.24±1.64	13.81±2.50	15.63±0.58	16.23±1.58 (+80.00)[b]
V	Methanolic extract of *Luffa*	14.92±0.82	9.31±1.50 (-37.58)[a]	11.95±1.34	14.32±1.67	15.54±0.39	16.16±0.86 (+73.11)[b]
a - Percentage variation compared to day 0; b - Percentage variation compared to day 2							

Table 5. Effect of Methanolic Extracts of *Momordica charantia* and *Luffa acutangula* on Haematocrit

Group	Treatment	Haematocrit (%) (Mean ± S.E.M) (%)					
		Day 0	Day 2	Day 7	Day 14	Day 21	Day 28
I	Normal Control	50.82±0.95	49.12±1.08	48.34±0.62	50.41±1.75	49.76±1.88	50.24±0.93
II	Anemic control	45.80±1.20	24.21±1.13 (-47.16)	27.66±1.43	30.20±1.31	32.81±1.71	34.93±1.51 (+44.21)
III	Dexorange syrup	45.03±0.93	27.60±1.62 (-38.66)	32.29±1.70	45.73±1.09	49.54±1.44	50.70±1.97 (+83.69)
IV	Methanolic extract of *Momordica*	42.71±1.53	27.46±1.27 (-35.83)	31.78±1.19	45.24±1.72	48.57±0.83	52.22±1.55 (+90.51)
V	Methanolic extract of *Luffa*	43.17±1.20	27.53±1.34 (-36.19)	33.02±1.88	45.70±1.03	48.26±1.28	49.14±2.55 (+78.54)
a - Percentage variation compared to day 0; b - Percentage variation compared to day 2							

Anti-Anaemic Activity of Methanolic Extracts

Vol. 16 (Supplementy Issue 2) 155 - 161, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3s.73

the methanolic extracts was compared with commercially used dexorange syrup. The dexorange syrup showed a significant increase of the content in haemoglobin after the first week of treatment.

Conclusion

From the above results, it was concluded that the methanolic extracts of *Momordica charantia* and *Luffa acutangula* showed promising anti-anaemic effect and among these two, *Momordica charantia* showed more efficacy. Further work is needed to be done on the isolation of specific phytochemical component that is responsible for anti-anaemic activity.

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Conflict of interest

The authors declare no conflict of interest.

Ethical Statement

The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India were followed and prior approval was soughted from Institutional Animal Ethics Committee (IAEC) for conducting the study (1529/PO/Re/11/CPCSEA./CHIPS/IAEC5/PRO-11/2016-17)

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Vol. 16 (Supplementy Issue 2) 155 - 161, October 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
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