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Research Article

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Muscle relaxant and antibacterial activity of leaf extracts of *Feronia limonia*

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ABSTRACT

Feronia limonia, commonly known as wood apple, has been used traditionally because of its medicinal values. In this study we evaluated the Muscle relaxant and antibacterial properties of the aqueous and ethanolic extracts of their leaves. The muscle relaxant activity was evaluated using rotarod method and actophotometer method. Thirty mice of both sexes were divided into five groups, with 1 group as control, 1 as standard (diazepam 5mg/kg i.p) and three test groups of aqueous extract (50mg/kg, 100mg/kg, 200mg/kg). Same procedure was repeated for ethanolic extract (50mg/kg, 100mg/kg, 200mg/kg). The results were statistically calculated using ANOVA. The results showed a significant reduction in the muscle coordination among the mice. Ethanolic extracts found to produce a better activity when compared to aqueous extract. Antibacterial activity was evaluated for 4 bacterial strains including 2 gram negative strains such as Pseudomonas aeruginosa, Escherichia coli and 2 gram positive strains such as Staphylococcus aureus, streptococcus faecalis. The zone of inhibition was analysed for both the extracts with ciprofloxacin 10mg as control. The zones were produced for a minimum dose of 25mg for both the extracts. All four strains were susceptible to both aqueous and ethanolic leaf extracts. Thus both these extracts showed muscle relaxant and antibacterial activities.

Key words: Muscle relaxants, Anti-bacterial, *Feronia limonia*.

INTRODUCTION

Feronia limonia is a tropical deciduous plant used in Ayurveda belonging to the family rutaceae. The leaves are dark-green and minutely toothed (Orwa C et.al 2009). The leaves are used for stomach ailments, astringent etc.(Parajapati ND et.al 2003). It is commonly known as wood apple and are well distributed across India. Leaf extracts of *Feronia limonia* had some anthelmintic activity (Orwa C et.al 2009). The important chemical constituent of leaf extracts of *Feronia limonia* were flavonoids, saponins and tannins (Ahamed et.al 2008).

Taxonomical classification(P Ghosh et.al 1982)

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Sapindales
Family : Rutaceae
Genus : Limonia

The present study focusses on the muscle relaxant and antibacterial properties of aqueous or ethanolic leaf extracts.

EXPERIMENTAL SECTION

Collection of materials:
The leaves of *Feronia limonia* were collected from a local herbarium in Chennai, Tamilnadu and the authentication was carried out in Dept. of Botany, Vels University. The leaves are shade dried.

ANIMALS:
Swiss albino mice of 20-25g weight of both sex were used. The animals were fed with pellet diet and maintained in standard conditions. The usage of animals has been approved by the Institutional Animal Ethics Committee XVI/VELS/PCOL/04/2000/CPCSEA/IAEC/25.11.14

Preparation of the extracts:
The leaves are pulverized and the coarse powder is subjected to soxhelation with 95% ethanol for the preparation ethanolic extract. The extract was concentrated using a rotary evaporator. The yield of 24.7% w/w was stored for the experimental purpose. The aqueous extract was prepared by the same procedure. The yield was found to be 27.2%w/w.

PHYTOCHEMICAL EVALUATION:
The aqueous and ethanolic extracts are subjected to the usual phytochemical screening of alkaloids glycosides, saponins, flavonoids, tannins, steroids, proteins, fats, carbohydrates and amino acids etc.

ACUTE TOXICITY STUDIES:
The acute oral toxicity study has been carried out as per the OECD 0423 guidelines with the help of 3 mice for each extract(OECD 2008).

ROTAROD TEST:
The skeletal muscle relaxant activity was studied using the rotarod apparatus. The animals were divided into 5 groups. Group 1 received normal saline, while group 2 received standard drug (diazepam (5mg/kg ip)). The other 3 groups received different doses of aqueous extract. The same procedure is followed for the ethanolic extract. The fall off time is noted with 25rpm(TB Al-Naggar et.al 2003).

LOCOMOTOR ACTIVITY:
The locomotor activity was studied using the actophotometer apparatus. The animals were divided into 5 groups. Group 1 received normal saline, while group 2 received standard drug (diazepam (5mg/kg ip)). The other 3 groups received different doses of aqueous extract. The same procedure is followed for the ethanolic extract. The basal activity of each animal is recorded. The percent decrease in activity is being noted(Vogel HG 2008)

STUDY DESIGN:
For muscle relaxant activity
Group I: normal saline
Group II: diazepam (5mg/kg ip)
Group III: 50mg of a/e leaf extract
Group IV: 100mg of a/e leaf extract
Group V: 200mg of a/e leaf extract
a/e – aqueous or ethanolic extract.

ANTIBACTERIAL PROPERTY:
Antibacterial property was assessed using agar plate diffusion method. Antibacterial activity was evaluated for 4 bacterial strains including 2 gram negative strains such as *Pseudomonas aeruginosa, Escherichia coli* and 2 gram positive strains such as *Staphylococcus aureus, streptococcus faecalis.* The zone of inhibition was analysed for both the extracts with ciprofloxacin 10mg as control. The zones were produced for a minimum dose of 25mg for both the extracts(Qureshi A A*et al* 2010).

STUDY DESIGN:
For anti-bacterial activity
Control – ciprofloxacin
Test sample I – 25mg of a/e leaf extract
Test sample II – 50mg of a/e leaf extract
Test sample III - 100mg of a/e leaf extract

STATISTICAL ANALYSIS:
Statistical analysis was carried out by using the one-way Analysis of Variance (ANOVA) followed by Dunnet's test. *P*-values < 0.05 were considered significant.

RESULTS

PHYTOCHEMICAL EVALUATION:

Table 1: Various phytochemical constituents present in these aqueous and ethanolic extracts

S.No	CONSTITUENT	AQUEOUS	ETHANOLIC
1	Carbohydrate	ABSENT	ABSENT
2	Alkaloids	ABSENT	ABSENT
3	Glycosides	ABSENT	ABSENT
4	Saponins	PRESENT	PRESENT
5	Phenols	ABSENT	ABSENT
6	Tanin	ABSENT	PRESENT
7	Flavanoids	ABSENT	PRESENT
8	Proteins & amino acid	PRESENT	PRESENT

ACUTE TOXICITY STUDIES:
The preliminary acute oral toxicity studies were carried out based on the OECD guidelines. No adverse effects have been seen upto 2000mg/kg for both the aqueous and ethanolic leaf extracts**.**

ROTA ROD TEST:

Table 2 : Aqueous extract

GROUP	FALL OFF TIME (SEC)	% DECREASE
G1 (NS)	245.4±9.098	-----
G2 (DIAZE)	12.34±4.33	94.72
G3 (50mg)	148.6±2.24**	39.58
G4 (100mg)	112.4±2.16**	55.21
G5 (200mg)	61.3± 6.11**	75.03

*n=6. **P<0.001*

table 2 shows the fall off time from rotarod as a measure of muscle relaxant activity of aqueous extract.

Table 3: Ethanolic extract

GROUP	FALL OFF TIME (SEC)	% DECREASE
G1 (NS)	245.4±9.098	-----
G2 (DIAZE)	12.34±4.33	94.72
G3 (50mg)	137.6±2.84**	44.94
G4 (100mg)	82.4±3.56**	66.43
G5 (200mg)	21.3± 5.01**	91.60

*n=6. **P<0.001*

table 3 shows the fall off time from rotarod as a measure of muscle relaxant activity of ethanolic extract

LOCOMOTOR ACTIVITY:

Table 4: aqueous extract

GROUP	5 MIN BEFORE	60 MIN AFTER	% REDUCTION
G1 (NS)	146±6.21	-----	
G2 (DIAZE)	143.2±2.66	11.6±1.2**	92.12
G3 (50mg)	145.4±2.19	102.42±3.5**	31.46
G4 (100mg)	149.5±3.22	72.21± 2.6**	53.20
G5 (200mg)	141.6±2.14	31.66± 5.4**	77.25

*n=6. **P<0.001*

Table 4 shows the % reduction in locomotor activity as a measure of muscle relaxant activity of aqueous extract.

Table 5: Ethanolic extract

GROUP	5 MIN BEFORE	60 MIN AFTER	% REDUCTION
G1 (NS)	146±6.21	-----	
G2 (DIAZE)	143.2±2.66	11.6±1.2**	92.12
G3 (50mg)	147.2±2.09	82.47±4.5**	44.08
G4 (100mg)	148.5±4.02	59.21± 2.8**	62.12
G5 (200mg)	144.6±2.74	15.46± 7.1**	90.04

*n=6. **P<0.001*

table 5 shows the % reduction in locomotor activity as a measure of muscle relaxant activity of ethanolic extract.

ANTIBACTERIAL ACTIVITY:

Table 6 : Zone of inhibition of various extracts on 4 species of bacteria

SPECIES	G+/G-	CONTROL	EXTRACT	25mcg/ml	50mcg/ml	100mcg/ml
Pseudomonas aeruginosa	Gram negative	15.3 ±0.33	Aqueous	2.2±0.45	4.7±0.66	8.8±0.29
			Ethanolic	3.9±0.99	7.1±0.38	15.8±0.43
Escherichia coli	Gram negative	16.4±0.67	Aqueous	1.4±1.2	2.6±0.47	5.4±0.74
			Ethanolic	3.6±0.44	7.4±0.55	16.6±0.56
Staphylococcus aureus	gram positive	19.8±0.87	Aqueous	4.1±0.65	7.9±0.88	18.6±0.67
			Ethanolic	4.9±0.55	9.9±0.72	21.4±0.77
streptococcus faecalis	gram positive	20.9±0.54	Aqueous	4.3±0.71	8.5±0.67	19.0±1.19
			Ethanolic	5.7±0.88	11.8±0.93	23.6±0.54

n= 4. All values are mean±SD in mm. p>0.005% are considered significant.

DISCUSSION

Feronia limonia is a rare plant to be used for medicinal purposes. The aqueous extracts and ethanolic extracts shows the presence of saponins, proteins and amino acids. The results correlate with the study done by Ghumarepramila et.al.(Ghumarepramila et.al 2013) .Acute toxicity studies that are carried out by OECD 0423 guidelines showed no toxicity upto the dose of 2000mg/kg. The presence of these compounds may be the reason for the muscle relaxant and antibacterial activities. Ethanolic extracts showed much better muscle relaxant activity than aqueous extract in both rotarod and photoactometer tests. The zones were produced for a minimum dose of 25mg for both the extracts. All four strains were susceptible to both aqueous and ethanolic leaf extracts. Gram positive organisms showed a much better inhibition than gram negative. Similarly ethanolic extracts were found to be superior.

CONCLUSION

On further recommendations, *Feronia limonia* can be exploited for medicinal purposes. This can provide as a natural substitute in treating bacterial infections and also as a topical muscle relaxant. This is recommended for further research for finding out the exact mechanism as well as to isolate the constituent.

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