Phytochemical Screening, Acute Toxicity and Anti-Nociceptive Activity of Methanol Stem Bark Extract of *Citrus aurantifolia* Linn

Mustapha A. Tijjani[1], Fanna I. Abdulrahman[2], Sherriff Adam[3], Usman B. Shamaki[4]

[1]*Department of Chemistry, Faculty of Science, University of Maiduguri, P.M.B 1069, Maiduguri, Borno State, Nigeria.*
[2]*Department of Science Laboratory Technology, Ramat Polytechnic Maiduguri, P.M.B. 1070 Maiduguri, Borno State – Nigeria.*
[3]*Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Maiduguri, Borno State., Nigeria*
[4]*Department of Pharmaceutical Chemisty, Faculty of Pharmacy, University of Maiduguri, Borno State, Nigeria*

Abstract: - Citrus aurantifolia **Linnhas many medicinal and pharmacological significance. This work aimed at evaluating the phytochemical constituents, acute toxicity and the anti-nociceptive activity of the methanol crude stem bark extract of *Citrus aurantifolia*. Presence of some bioactive metabolites: cardiac-glycosides, terpeniods, flavonoids, tannins and phlobatannins were found in crude methanol extract. Acute toxicity (LD_{50}) of the methanol crude extract was determined and it was observed that there was no mortality at 5000mg/kg b.d.wt. Hence, the extract was found to be non toxic. In acetic-acid induced test the methanol stem bark extract demonstrated significant antinociceptive activities (P≤0.05), the percentage inhibitions of the treatment groups were 48.3%, 52.6% and 57.9%.Pentozocine (20 mg/kg), a standard drug gave the percentage inhibition of 67.8%. The extract on tail immersion test appeared to increase the mean reaction time at (30 min.) were found to be 5.00±0.00s, 5.80±0.37s, 6.00±0.32s and 7.00±0.32s respectively and at (120 min.), were observed to be 4.40±0.25s, 5.20±0.20s, 5.40±0.25s and 5.80±0.20s. Pentozocine gave mean reaction time of 9.00±0.32s, 8.00±0.32s, 7.60±0.68s and 6.20±0.20s respectively at various post treatment time intervals.The percentage inhibitions of methanol extract on tail immersion test at dose of 400mg/kg was 20%,at 30min, at 60min, was 71.4%, at 90min. was 32% while at 120min. was 22.7% and finally, for the 600mg/kg body weight were 40%, 80.9%, 36% and 31.8 respectively. The pentozocine gave percentage inhibitions at 30, 60, 90, 120 min were 80%, 90%, 52% and 40.9% for various doses at post treatment time were found to be higher than that of the methanolextract. Moreover the extract appeared to induced an increase in pain threshold at (P<0.05) to Eddy's hot plate. The maximum dose of 600mg/kg, the percentage increase in pain threshold of 82.4% respectively. Pentozocine (20 mg/kg) showed mean reaction time of 6.60±0.24s and percentage increase in pain threshold 94.1%. The activity of the pentozocine was found to be significantly higher compared to the extract although, they were all significant at (P≤ 0.05) across the column. Thus, the usage of the plant in treatment of analgesic condition by local people could be justified.**

Key words: **Citrusaurantifolia Phytochemistry Plants, Analgesics Pharmacology**

I. INTRODUCTION

Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diversity types of plants grow in different parts of the country. In Nigeria, thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Herbal medicine is still the mainstay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents [1]. Plants with their complex chemical storehouse of biodynamic compounds serve as plant defense mechanisms against inversion by microorganisms and insects and can provide valuable sources of natural antibacterial agents [2,3]. The active principles isolated from plants appeared to be one of the important alternatives, when compared to many sub-standard orthodox synthetic medicines, because of their less or no side effects and better bio-availability[4,5]. *Citrus* fruits, which belong to the family of *Rutaceae* are one of the main fruit tree crops grown throughout the world. Although sweet orange (*Citrus sinensis*) is the major fruit in this group accounting for about 70% of citrus output. The group also encompasses small citrus fruits such as tangerine tree (*Citrus reticulata*), grapefruit tree (*Citrus vitis*), lime tree (*Citrus aurantifolia*) and lemon tree (*Citrus limonum*) [6,]. Limes (*Citrus aurantifolia*) are the fruits of tropical citrus tree closelyrelated to lemons because of their distinctive flavor. Flowers and fruit appear throughout the year but are most abundant from May to September.

This work was undertaken to investigate the phytochemical constituents, acute toxicity and anti-nociceptic activity of crude extract of methanol stem bark of *C. aurantifolia* so as to verify or otherwise the traditional claimed on the uses of the plant parts by traditional herbalists.

II. MATERIALS AND METHODS

Sample collection and Extraction

The stem bark of *C. aurantifolia* was collected from Damboa road, Maiduguri, Borno State. The plant part was authenticated by a botanist in the Department of Biological Sciences, University of Maiduguri, Borno State. The plant was dried under shade and pulverized into powder.Thepowdered plant material (200g) was soaked in with methanol for three days with frequent agitation. The extract was filtered, concentrated with rotary evaporator. The methanol extract was screen for phytochemical and anti-nociceptive activity.

Phytochemical Analysis

The methanol extract was subjected to qualitative phytochemical screening using standard procedures described byBrain and Tuner,[7,8,9].

Extract Preparation

Methanol crude stem bark extract of *C. aurantifolia*(2 g) was dissolved in 10 ml distilled water, to give a stock solution of 200 mg/ml.

Acute Toxicity Test

The method of Lorke (1983)[10] was used for this study. Ninerats of both sexes were randomlygrouped into threeand were treated with 10, 100 and 1000 mg/kg of the methanolic extract intraperitoneally in Phase I. The animals were then given free access to feed and water. They were observed over a period of 24 hours for signs of toxicity and mortality. Three groups of one rat each for phase II were used by using the same method. No sign of toxicity and mortality wasobserved.

Antinociceptic Evaluation

Tail Immersion Test

The tail immersion method was used to evaluate the central mechanism of analgesic activity [11].This was based on the method described by [12].Twenty five albino rats of both sexes were randomly divided into five groups (A, B, C, D and E) of five rats each. Theywere deprived of food for twenty hours before the commencement of the experiment. Those in group A (negative control)received distilled water (10 ml/kg) while those in groups B, C and D received 200, 400 and 600 mg/kg respectively of extract whilst those in group E (positive control) received pentazocine (20 mg/kg). All treatments were byintraperitoneally(i.p.) route. Thirty minutes later, the tail (upto 10 cm) was dipped into a water bath maintained at 55 ± 0.5 °C. Thetime (in seconds) to withdraw the tail clearly out of the water was taken as the reaction time. The latent period ofthe tail response was determined at 30, 60, 90 and 120 min after the administration of drugs and extract.

Thepercentage (%) increase in pain threshold (latency) was calculated by the formula below;

$$\%IPT=\frac{Mean\ reaction\ time\ of\ test\ group-Mean\ reaction\ time\ of\ negative\ control\ group}{Mean\ reaction\ time\ of\ negative\ contro\ l\ group}\times 100$$

(IPT)= Increase in Pain Threshold

Effect of the Extract on Hot plate Test

The method described by[13] as modified by [14] was used for this study. Albino rats of both sexes were randomly grouped into five groups (A,B,C,D,E) of five rats each, fasted for 12 hours. The rats were then treated as follows: Group A were treated with 10 ml normal saline (negative control), groups B, C, and D were received 200, 400 and 600 mg/kg of *Citrus aurantifolia* extract respectively. Group E was treated with 20 mg/kg of pentozocine (positive control). Thirty minutes after drug and extract administration, the pain reaction time for each rat was determined and recorded respectively.Each of the rats was placed on a hot plate maintained at the temperature of 55 ± 1 C and the pain reaction time (PRT) or latency period determined with a stop watch was recorded and represents the time taken for the rat to react to the pain stimulus. The response to pain stimulus considered included jumping, raising and licking of hind foot. The cut off time was fixed for 20 seconds.

Effect of the Extract on Acetic Acid Induced Writhes Test

This study was carried out using the method of Koster *et al*, (1995) [15] as modified by Danbisya and Lee (1999) [20]. Twenty five albino mice of both sexes were randomly divided into five groups (A-E) of five mice per group. They were fasted for 12 hours and later treated as follows: Group A mice received normal saline 2 mg/kg (negative control group), Group B, C, and D received 100, 200, 300 mg/kg of *citrus aurantifolia* extract while group E was treated with 20mg/kg body weight of pentozocine (positive control group) respectively all by gastric gavage. One hour (1hr) after administration of drug and extract, 0.7% glacial acetic acid (10 mg/kg) was given intraperitoneally (i.p.) to all of the mice to induce pain characterised by abdominal constrictions or writhes. The number of writhes observed in each mouse were counted for 30 minutes and recorded. The percentage protection against abdominal writhing was used to assess the degree of analgesia and was calculated using the formula [16].

$$\%INHIBITION=\frac{meanno\ .ofwri\ htes\ innegativeconrolgroup\ -meanno\ .ofwrit\ hesintestgroup}{meanno\ .ofwrit\ hes\ innegativecontrol\ \ group}\times 100$$

The number of writhing movements that occurs was counted for 10 minutes after 5 minutes latency period for each animal.

Data Analysis

Results were expressed as Mean±S.E.M (n=1). The inhibition was compared by using one way analysis of variance(ANOVA) using graph pad prism, version 4.0 (Graph pad, San Diego, CA, USA). P value ≤ 0.05 was considered significant.

III. RESULTS

Table 1.Preliminary Phytochemical Screening of crude methanol and partitioned portions of stem bark extract of C*itrus aurantifolia* Linn.

S/No	Test	MCE	NHP	EAP	NBP	AQP
1.	Test for Carbohydrates					
i	General test-molish test					
ii	Test for monosaccharide-Barfoed test	+		-	-	+
iii	Test for Free reducing sugars-Fehlings	+		-	-	+
iv	Test for Combined reducing sugars	+	-	-	-	+
v	Test for Pentoses	+	-	-	-	-
vi	Test for Ketoses	+	-	+	-	-
vii	Test for soluble Starch	+	-	-	-	+
2i.	Test for Anthraquinones	-	-	-	-	-
ii	Test for Combined Anthraquinones	-	-	-	-	-
3.	Test for Cardiac-glycosides					
i.	Salkowski's Test	+	+	-	+	-
ii.	Liebermann-Burchadr's test	+	+	+	+	+
4.	Test for Terpenoids	+	+	+	+	-
5.	Test for Flavonoids					
i	Shinoda's test	+	+	+	+	-
ii	Ferric Chloride test	+		+	+	+
iii	Lead acetate test	-		-	-	-
iv.	Sodium hydroxide test	+		-	-	-
6.	Test for Saponins					
i.	Fronthing test	-		-	-	-
7.	Test for Phlobatannins	+		-	-	-
8.	Test for tannins	-		-	-	-
i.	Ferric Chloride Test	+				
ii.	Lead acetate test	-		-	-	-
10	Test for Alkaloids					
i	Drangendroff's reagent	+	-	-	-	+
i	Mayer's reagent	+	-	-	-	+

The results of phytochemical screening of the methanol crude stem bark extract of *Citrus aurantifolia* as shown in table 1 indicates that the plant contain many secondary metabolites. These include carbohydrates, cardiac-glycosides, terpeniods, flavonoids, tannins and phlobatanninshowever, anthraquinones and saponins were not found in crude methanol stem bark extract

Table 2.Acute toxicity studies (LD_{50}) of Methanol crude stem bark extract of *Citrus aurantifolia* Linn.

S/No.	Phase	No. of Rats	Dose(mg/kgbd.wt.)	Clinical Sign	Mortality
1.	1	3	10	None	0/3
2.	1	3	100	None	0/3
3.	1	3	1000	None	0/3
4.	2	1	1600	None	0/1
5.	2	1	2900	None	0/1
6.	2	1	5000	None	0/1

No mortality was observed at 5000mg/kg body weight in acute toxicity studies as shown in table 2 and this shows that thecrude methanol extract of *Citrus aurantifolia* has low toxicity

Table 3. Effect of Methanol crude stem bark extract of *Citrus aurantifolia* Linnon Acetic acid-induced writhing in mice

		Mean±SEM	
S/NO.	Treatment (mg/kgbd.wt.)	Number of writhing/(s)	% Inhibition
1	Normal saline	64.60±0.51	-
2	100	33.40±0.40*	48.3
3	200	30.60±0.40*	52.6
4	300	27.20±0.37*	57.9
5	Pentozocine(20)	20.80±0.37*	67.8

The methanol stem bark extract demonstrated significant antinociceptive activities ($P \leq 0.05$), at doses of 100, 200 and 300 mg/kg body weight of the extract and the mean number of writhing movement were found to be 33.40±0.40 s, 30.60±0.40 s and 27.20±0.37 s respectively at shown in table 3. The percentage inhibition of the treatment groups were 48.3%, 52.6% and 57.9% which showed dose dependent effects across the column (Table 3 and 4). The pentozocine (20 mg), a standard drug had mean number of writhing movement of 20.80±0.37 s with the percentage inhibition of 67.8%. The activity is more pronounced at a higher dose of 300 mg/kg which gave the highest percentage inhibition (57.9%) of the abdominal constriction induced by acetic acid. This was found to be significantly lower than pentozocine (20 mg/kg) in the extent to which writhing or stretching was reduced

Table 4.Effect of Methanol crude stem bark extract of *Citrus aurantifolia* Linn on Hot-plate test in Wister albino rats

			Mean±SEM	
S/NO.	Treatment	Dose(mg/kgbd.wt.)	Time(s)	% Increase in Pain threshold
1	Normal saline	10	3.40±0.24	-
2	MCE	200	4.00±0.32*	17.6
3	MCE	400	5.00±0.32*	47.1
4	MCE	600	6.20±0.20*	82.4
5	Pentozocine(20)	20	6.60±0.24*	94.1

At a dose of 200 mg/kg, the mean reaction time was 4.00±0.32s with percentage increase in pain threshold of 17.6%.While at a dose of 400 mg/kg, the mean reaction time was 5.00±0.32s and percentage increase in pain threshold (47.1%) as shown in Table 4. Lastly the maximum dose of 600 mg/kg the mean reaction time was 6.20±0.20s had percentage increase in pain threshold of 82.4% respectively. Pentozocine (20 mg/kg) showed mean reaction time of 6.60±0.24s and percentage increase in pain threshold 94.1%

Table 5.Effect of Methanol crude stem bark extract of *Citrus aurantifolia* Linn on Tail immersion test of Wister albino Rats

			Mean±SEM/ (S)			
S/No.	Treatment	Dose(mg/kgbd.wt)	30 min.	60 min.	90 min.	120 min.
1	N.saline	10	5.00±0.00	4.20±0.37	4.80±0.20	4.40±0.25
2	MCE	200	5.80±0.37	5.80±0.49*	6.00±0.00*	5.20±0.20*
3	MCE	400	6.00±0.32	7.20±0.49*	6.60±0.25*	5.40±0.25*
4	MCE	600	7.00±0.32*	7.60±0.51*	6.80±0.37*	5.80±0.20*
5	Pentozocine	20	9.00±0.32*	8.00±0.32*	7.60±0.68*	6.20±0.20*

Table 5 here shows the effects of methanol crude stem bark extract of *Citrus aurantifolia* on tail immersion test of Wister albino rats. The mean reaction times at (30 min.) were found to be 5.00±0.00s, 5.80±0.37 s, 6.00±0.32s and 7.00±0.32 s respectively. Like-wise at (60min.), were 4.20±0.37s, 5.80±0.49 s, 7.20±0.49 s and 7.60±0.51 s. While at (90 min.) the mean reaction times were 4.80±0.20s, 6.00±0.00 s, 6.60±0.25 s and 6.80±0.37 s. Finally mean reaction time at (120 min.) were observed to be 4.40±0.25s, 5.20±0.20s, 5.40±0.25s and 5.80±0.20s Pentozocine, a standard analgesic drug gave mean reaction time at various time intervals (30, 60, 90 and 120 min.) were found to be 9.00±0.32 s, 8.00±0.32 s, 7.60±0.68 s and 6.20±0.20 s respectively

Table 6.Percentage Inhibitions of Methanol crude stem bark extract of *Citrus aurantifolia* on Tail immersion test in Wister albino rats

S/No.	Treatment	Dose (mg/kgbd.wt.)	%Inhibition (30min.)	%Inhibition (60min.)	%Inhition (90min.)	%Inhibition (120)
1	N.saline	10	0.0	0.0	0.0	0.0
2	MCE	200	16	38	20	18.1
3	MCE	400	20	71.4	32	22.7
4	MCE	600	40	80.9	36	31.8
5	Pentozocine	20	80	90	52	40.9

Percentage inhibitions at dose of 200 mg/kg, at the various post treatment time. At 30min. was 16%, 60min. was 38%, 90min. was 20% while at 120min. was 18.1% respectively. Like- wise the percentage inhibitions for dose of 400 mg/kg, at 30min. was 20%, at 60min. was 71.4%, at 90min. was 32% and 22.7% at 120min. Finally, for dose of 600 mg/kg body weight at 30min., 60min., 90min., 120min. were 40%, 80.9%, 36% and 31.8 respectively. The pentozocine (20 mg/kg) gave percentage inhibitions at 30, 60, 90 and 120min. were found to be 80%, 90%, 52% and 40.9% respectively

IV. DISCUSSIONS

The results of preliminary phytochemical screening of the methanol crude stem bark extract of *Citrus aurantifolia*as shown in table 1 indicates that the plant contain many secondary metabolites. The presence of secondary metabolites such as carbohydrates, cardiac-glycosides, terpeniods, flavonoids, tannins and phlobatannins were observed in the extracts however, anthraquinones, alkaloids and saponins were not found in crude methanol stem bark extract.These compounds have been known to exert pharmacological and antagonistic effects and still some are capable of protecting the active ingredient in herbs from decomposing either chemically or physiologically [17] Flavonoids are reported to exhibit several biological effects such as antihepatotoxic, anti-inflammatory and antiulceractivity [18.19]. They (flavonoid) are potentanti-oxidants and have free radical scavenging abilities[20].Many have anti-allergic, antiviral actions and some of them provide protection against cardiovascular mortality [21,22] Also flavonoid hasshown to inhibit the growth of various cancer cell lines in vitro and reduce tumor development in experimental animals [23] Several flavonoids such as catechin, apigenin, quercetin, naringenin and rutin, are reported for their heptoprotective activities [24] .

There was no mortality was observed at 5000mg/kg body weight in acute toxicity studies andthis shows that thecrude methanol extract of *Citrus aurantifolia* has low toxicity as shown in table 2. This result is in consonance with the findings of Baars*et al.* [25].

The methanol stem bark extract demonstrated significant antinociceptive activities ($P \leq 0.05$), at doses of 100, 200 and 300 mg/kg body weight of the extract and the mean number of writhing movement were found to be 33.40±0.40s, 30.60±0.40s and 27.20±0.37s respectively at shown in table 3. The percentage inhibition of the treatment groups were 48.3%, 52.6% and 57.9% which showed dose dependent effects across the column (Table 3 and 4). The pentozocine (20 mg), a standard drug had mean number of writhing movement of 20.80±0.37s with the percentage inhibition of 67.8%. The activity of the extract is more pronounced at a higher dose of 300 mg/kg which gave the highest percentage inhibition (57.9%) of the abdominal constriction induced by acetic acid. This was found to be significantly lower than pentozocine (20 mg/kg) in the extent to which writhing or stretching was reduced. Writhing induced by chemical substances such as acetic acid injected intraperitoneally are due to sensitization of nociceptors by prostaglandins [26,27] and test is useful for the evaluation of mild analgesic induced by non-steroidal anti-inflammatory compounds [28,29].

Effects of methanol crude stem bark extract of *Citrus aurantifolia* on tail immersion test of Wister albino rats. The mean reaction times at 30 min. were found to be 5.00±0.00s, 5.80±0.37s, 6.00±0.32s and 7.00±0.32s respectively. Like-wise at 60min., were 4.20±0.37s, 5.80±0.49s, 7.20±0.49s and 7.60±0.51s. While at (90 min.) the mean reaction times were 4.80±0.20s, 6.00±0.00s, 6.60±0.25s and 6.80±0.37s. Finally mean reaction time at (120 min.) were observed to be 4.40±0.25s, 5.20±0.20s, 5.40±0.25s and 5.80±0.20s as shown in Tables 4 and 5. Pentozocine, a standard analgesic drug, gave mean reaction time at various time intervals (30, 60, 90 and 120 min.) were found to be 9.00±0.32s, 8.00±0.32s, 7.60±0.68s and 6.20±0.20s respectively. Percentage inhibitions at dose of 200 mg/kg, at the various post treatment time. At 30min. was 16%, 60min. was 38%, 90min. was 20%

while at 120min. was 18.1%. Like- wise the percentage inhibitions for dose of 400 mg/kg, at 30min. was 20%, at 60min. was 71.4%, at 90min. was 32% and 22.7% at 120min. Finally, for dose of 600 mg/kg body weight at 30min., 60min., 90min., 120min. were 40%, 80.9%, 36% and 31.8. The pentozocine (20 mg/kg) gave percentage inhibitions at 30, 60, 90 and 120min. were found to be 80%, 90%, 52% and 40.9% respectively (Tables 5 and 6). The activity of the standard drugs was found to be significantly higher compared to the extract. The crude methanol stem bark extract of *Citrus aurantifolia* at post-treatment times induced a dose dependent increase in pain threshold (latency) compared to the control. This is in line with the findings of Abdulrahman*et al* [30].

The result shows that the crude methanol stem bark extract of *Citrus aurantifolia* Linn appeared to induced an increase in pain threshold at ($P<0.05$) to Eddy's hot plate(Table 4). At a dose of 200 mg/kg, the mean reaction time was 4.00±0.32s with percentage increase in pain threshold of 17.6%.While at a dose of 400 mg/kg, the mean reaction time was 5.00±0.32s and percentage increase in pain threshold (47.1%). Lastly the maximum dose of 600 mg/kg the mean reaction time was 6.20±0.20s had percentage increase in pain threshold of 82.4% respectively. Pentozocine (20 mg/kg) showed mean reaction time of 6.60±0.24s and percentage increase in pain threshold 94.1%. The activity of the pentozocine was found to be significantly higher compared to the extract although, they were all significant at ($P\leq 0.05$) across the column.

The anti-nociceptive effects observed for the methanol crude extract were comparable to that of the positive control group (pentozocine) this might be considered as an indication of anti-nociceptive property of the extract. The remarkable anti-nociceptive effects observed may be due to the presence of some bioactive metabolites detected in crude methanol stem bark extract of *Citrus aurantifolia* such as cardiac-glycosides, terpenoids, flavonoids, tannins and phlobatannins. Flavonoids have been reported to play roles in analgesic activity primarily by targeting prostaglandins and tannins also haz been reported to have analgesic activity[31].

V. CONCLUSIONS

1. The results of phytochemical screening of the methanol crude stem bark extract of *Citrus aurantifolia* indicates that the plant contain many secondary metabolites such as carbohydrates, cardiac-glycosides, terpeniods, flavonoids, tannins and phlobatannins however, anthraquinones and saponins were not found in crude methanol stem bark extract
2. The methanol stem bark extract is found to be non-toxic
3. The methanol stem bark extract has significant antinoceiptive activity as shown in hot plate induced and tail immersion test
4. This study supports the local usageof stem bark *Citrus aurantifolia* for medicinal purpose.

ACKNOWLEDGEMENT

The authors wish to acknowledge the support and funding from Tertiary Education Trust Fund (TETFUND) and Messrs.' Fine Akawu of Chemistry Department and Bitrus Akawu of Veterinary Toxicology and Pharmacology all of University of Maiduguri,Borno State, Nigeria

REFERENCES

[1]. Akerele, O. (1993). Summary of WHO Guidelines for the Assessment of Herbal Medicines. Herbal Gram.**22**:13-28.
[2]. Abel, C. and Busia, K. (2005). An Explanatory Ethnobotanical Study of the Practice of Herbal Medicine by the Akan people of Ghana. *Altern.Med.* Rev. 10:112-122.
[3]. Roosita, K., Kusharto, C. M., Sekivama, M., Fachrurozi, Y., and Ohtsuka, R. (2008). Medicinal plants used by the villagers of a Sundanese community in west Java, Indonesia **115**:72-81.
[4]. Scazzochio, F., Cometa, M., Tomassin, L. and Palmery, M. (2001). Antibacterial Activity of Hydrastis Canadensis Extract and its Major Isolated Alkaloids.*Panta.Med.* **67**:561-564.
[5]. Joshi, B. Sah, G. B.,Basnet, B. B., Bhatt, M. Sharma, R.,Subedi, K., Pandey, J. and Malla, R. (2011). Phytochemical Extraction and Antimicrobial Properties of Different Medicinal Plants: Ocimum sanctum (Tutsi), *Eugenia caryophyllata* (Clove), Achyranthesbidentata (Datiwan) and *Azadirachtaindica* (Neem*). J. Microbiol. Amtimicrob.***3**(1),1-7.
[6]. Okwi, D. E. and Emenike, I. N. (2006). Evaluation of the Phytonutrients and Vitamins Contents of *Citrus* Fruits. *Intern. J. Mol. Med. and Adv. Sci.***2**(1): 1-6.
[7]. Brain, K. R. and Tuner, T. D. (1975). The Practical Evaluation of Pharmaceutical Weight ScienceTechnical Bristol, Britain.140-144; 152-154.
[8]. Markham, K. R. (1982). Techniques of Flavonoids Identification.*Academic Press*, London.193-204.
[9]. Trease, G. E. and Evans, W. C. (2002).Textbook of Pharmacogonosy, 14th Edition. W.B Saundors Company LTD. 24-25 Oval, London. NW7DX, UK and printed by Harcourt Brace and Company. 13-53, 117-139.
[10]. Lorke, D. (1983). A New Approach to Practical Acute Toxicity.Archives of Toxicology; **53**:275-289.
[11]. Ramabadran, K., Banisnath.,MTurndorf, H., and Puig, M. M (1989). Tail immersion test for the evaluation of a nociceptive reaction in mice: methodological considerations of *J.Pharmacol.toxicol. Methods,***21**: 21-31.
[12]. Singh, S. and Majumdar, D. K. (1995). Analgesic activity of *Ocimum sanctum* and its possible mechanism of action.*Int. J. Pharmacogn.,* **33**, 188-192.
[13]. Shetty, S. N. and Anika, S. M. (1982). Laboratory Manual of Pharmacology and Toxicology, 1st ed. Enugu: Fourth Dimension Publishers.pp.218-221.
[14]. Franzotti, E. M., Santos, C.V .F., Rodrigues, H. M. S., Mourao, R. H. V. and Andrade, M. R. (2000).Anti-inflammatory, Analgesic and Acute Toxicity of *Sida CordiforliaL. J.Ethnopharmacol*.,**72**:273-277.
[15]. Koster, R., Anderson, M. and Debeer, E. J. (1995).Acetic acid for Analgesic Screening. Federation Proceedings; **18**: 412-415.
[16]. Dambisya, Y. M. and Lee, S. (1999). Influence of Temperature, pH and Naloxone on the Anti- nociceptive Activity of *Chanastriatus*(Haraun) Extract in Mice. *J Ethnopharmacol*; **66**: 181-186.
[17]. Bolon, B, and St. Omar, V. E. V. (1989). Behavioral and development effects in sucking mice following material exposure to the mycotoxin Secaconic acid. *J. Pharmaco., and Biochem.of Behav*, **34**:229-231.

[18]. Bors, W., Heller, W., Michel, C. and Saran, M. (1990). Flavonoids as antioxidants: Determination of radical scavenging efficiencies. *Methods in Enzymology*, **186**:343-355.

[19]. Colerige Smith, P. O., Thomas, P., Scurr, J. H. and Dormandy, J. A. (1980). Causes of various ulceration, a new hypothesis, *Brit. Med. J.*,**296**:1726-1727.

[20]. Kumar, S., Mishra, A. and Pandey, K. (2013). Antioxidant mediated protective effect of *Partheniumhysterophorus* against oxidative damage using in vitro models. "BMC *Complementary and Alternative Medicine*, 13(120): 34-42.

[21]. Clack, W. and Mackay, E. (1950). Effect of flavonoid substances on histamine toxicity, anaphylactic shock and histamine enhanced capacity to dye. *J. Aller.*, **21**:133-147.

[22]. Hertog, M. G. L., Fesken, E. J. M., Holiman, P. C. H., Kantan, M. B. and Kromhout, D. (1993).Dietray Antioxidants; Falconoid and Risk of Coronary Heart Diseases. The Zutphen Elderly Study *Lancet*, **342**, 1007-1011.

[23]. Mori, A., Nishinoc, E. N and Tavata, S. (1988). Cytotoxicity of plant flavonoids against Hela cells. *Phytochemistry*,**27**: 1017-20.

[24]. Tapas, A. R., Sakarkar, D. M. and Kakde, R. B. (2008). Flavonoids as nutraceuticals: A review. *Tropical J. Pharm. Res.*, **7**: 1089-1099.

[25]. Baars, E. W., Jong, M., Nierop, A. F. M., Boers, I. and Savelkoul, F. H. J. (2011) Citrus/Cydonia Compositum Subcutaneous Injections Versus nasal Spray for Seasonal Allergic rhinitis: *A Randomized Controlled Trial on Efficacy and Afety.* ISRN Allergy; doi:**10**. 5402/2011/836051

[26]. Nunez, G. M., Emim, J. A., Souccar, C., &Lapa, A. J. (1997). Analgesic And Anti-inflammatory Activities of the Aqueous Extract of *Plantago major* L. *Int. J. Pharmacogn.*, **35**(2): 99-104.

[27]. Yongna, Z., Wapana, R., Pisit, B., Zhongkun, L. and Rongpin, Z. (2005). Analgesic and the Antipyretic Activities of the Aqueous Extract of *Urtica* macrorrhiza in Experimental Animals.*Fitoterapia*.**75**, 91-95.

[28]. Ferreira, S. H., & Vane, J. P. (1974). New Aspect on the Mode of Action of Non-steroidal Anti-inflammatory Drugs. *Ann.Rez.Pharmacol.*,**14**, 57

[29]. Eerkenkopf, J. W., and Waionmann, B. M. (1988). Production of Prostaglandins in Mice Following Intraperitoneal Injection of Acetic acid, Phenylbenzoquinone and Zymosan: its role in the Writhing Response. *Prostaglandins*.**36**: 698-709.

[30]. Abdulrahman, F. I., Sandabe, U. K. and Iguniwei, P. B. (2005). Anticonvulsant, antinociceptive and antipyretic effects of aqueous extract of *Terminaliaavicennoides* stem bark on rats, *Sahel Journal of Veterinary Science*, **4**(1): 43-47.

[31]. Sodipo, O. A., Mamza, U. T., Abdulrahman, F. I. And Khan, I. Z. (2015). Evaluation of Phytochemical, Antimicrobial and Toxicity Studies of Ethanolic Stem bark extract of *phyllantusAmarus Thonn*and*Schum (Euphorbiacea), TheInl. J. Scie. And Tech*.**3**(6): 262-268