Scholars Academic Journal of Pharmacy (SAJP) **ISSN 2320-4206 (Online)**
Sch. Acad. J. Pharm., 2017; 6(9): 403-409 **ISSN 2347-9531 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Evaluation *In-Vitro* Antioxidant, Antimicrobial and Laxative Potential of Ethanol Leaves Extract of *Synedrella nodiflora* (L) Gaertn on Wistar Albino Rats

M.A Jimoh[1], O.D Omodamiro[2]
[1]Department of Plant Science & Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia-state, Nigeria
[2]Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia-state, Nigeria

***Corresponding author**
M.A Jimoh

Article History
Received: 03.09.2017
Accepted: 09.09.2017
Published:30.09.2017

DOI:
10.21276/sajp.2017.6.9.5

Abstract: *Synedrella nodiflora* (L) Gaertn, commonly known as Pig grass, Cinderella weed, node weed is a species of flowering plant in the family of Asteraceae. It is a medicinal plant mostly used in the herbal medicine to cure leprosy, swelling, constipation, dysentery and diarrhea. Ethanol leaves extract of *Synedrella nodiflora* were investigated on in-vitro antioxidant, antimicrobial and laxatives activities in albino rats and were compared with the standard drugs Ascorbic acid(100mg/kg), Ciprofloxacin(40mg/kg) and Ducox(300mg/kg) respectively. The extract were administered based on high and low doses, 2000,1000,500,250,125 and 62.5mg/ml. Different models such as DPPH radical scavenging activity, Nitric oxide scavenging activity and Anti-lipid peroxidation activity were used to determine antioxidant activity. Healthy adult wistar albino rats of both sex weighing 150-200g was used to determine laxative effect of the ethanol leaves extract, for the antimicrobial activity, clinical strains of microorganism used for the study includes*: E. coli, Proteus mirabilis, Pseudomona aeruginose, Staphylococcus aureus, Salmonella typi and Klebsiella pneumonia*. The agar disc diffusion method was used and zone of inhibition recorded also minimum inhibitory concentration was determined. Result, the IC_{50} for nitric oxide yielded 70.70+- 3.52 at 200ug/ml, for anti-lipid peroxidation it yielded 53.97+-5.08 at 100ug/ml, but for DPPH, the results were less when compared with the standard drug(Ascorbic acid). The antimicrobial activity results at different graded concentrations of 2000, 1000, 500, 250, 125 and 62.5mg/ml, showed that the ethanol extract containing 2000mg/ml has the highest mean+-SD zone of inhibition against the clinical pathogens while the disc containing 62.5mg/ml revealed least inhibitory zones. The laxatives activity, there was a dose dependent increase in the faecal output of the rats when compared to the control group. The effect of the extract shows a similar trend to that of the standard drug which justified its medicinal potency.
Keywords: *Syndrella nodiflora*, Antimicrobial, Nitric oxide, DPPH, Lipid peroxidation, Laxative.

INTRODUCTION

It has been estimated that up to 80% of the population of Africa uses traditional medicine in primary health care. Approximately 25% of all prescription drugs used contain one or more bioactive compounds derived from plants [1]. Yet fewer 10% of the plants species have been examined for the presence of bioactive compounds [2]. Hence screenings of antimicrobial plants for new bioactive agents as alternative to conventional antibiotic drugs possess an enormous challenge and are important especially with the emergence of drug resistant disease strains [3]. Such plant products are considered as biodegradable and have fewer side effects than conventional antimicrobial drugs [4].

Synedrella nodiflora(L) Gaertn belongs to the family *Asteraceae*. It is a small common annual weed of waste places and found along the banks of rivers, streams and also along roadsides [5]. In Nigeria, the plant is used to treat cardiac distresses and to stop wound bleeding [6]. The hydro-ethanoic extract of the whole plant has been found to possess anticonvulsant [7], Sedative [8]. For over thousands of years now, natural plants have been seen as a valuable source of medicinal agents with proven potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents.

Fig-1: *Synedrella nodiflora*

Laxative are foods or drugs that increases bowel movement, laxatives contains chemicals that help increase stool motility, bulk and frequent relieving of temporary constipation. Antioxidants comprises of a range of substances that play a role in protecting biological system against the deleterious effect of oxidative processes on macromolecules such as proteins, lipids and carbohydrate. This scientific study is set to conform the antioxidant, antimicrobial and laxative potential of *S. nodiflora*.

MATERIALS AND METHODS

Collection of Plant material

Fresh leaves of *S. nodiflora* were harvested from st. Stephen boys academy international Ehimmiri housing estate Umuahia, Abia State (5.55^0N, 7.4922^OE)and was authenticated by a Taxonomist (Dr. Mulikat Abiola Jimoh) of the department of Plant Science & Biotechnology, Michael Okpara University of Agriculture, Umudike. A voucher specimen (Herbarium No. 1389) was deposited in the Herbarium of the department of Plant Science & Biotechnology, College of Natural Sciences. The leaves were rinsed severally with clean tap water to remove dust particles and debris and thereafter allowed to drain completely.

Preparation of Extract

The plant was air dried in the plant house of Michael Okpara University of Agriculture, Umudike for 4days, the dry leave were taken for pulverization by a pulverizing machine in the department of Soil Science, National Root Crop Research Institute, Umudike. 200g of the powder was obtained and 100g of the powder was soaked in 400ml of 95% ethanol for a period of 72hrs. The resulting aqueous mixture was then filtered with Whitman No.1 filter paper to obtain the filtrate. The filtrate was then allowed to evaporate; 8.2g was obtained as residue and was stored in a refrigerator at 4^oC.

Experimental Animals

Twenty-four (24) albino rats (*Rattus norregicus)* weighing between 150-200g of both sexes and mices were obtained from the animal house of University of Nsukka. The animals were harboured in animal house in the department Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike in clean metabolic cages under standard laboratory condition of 12hrs light/dark cycle. They had free access to vital pellet feed and clean water as they were allowed to acclimatize for 3weeks.

Acute Toxicity

The acute toxicity of the extract was done using sixteen mice of both sex divided into 8groups, each group have 2mice. Group A mice received intra-peritoneally 8000mg/kg, while group B, C, D, E, F, G and H received 7000, 6000, 5000, 4000, 3000, 2000 and 1000mg/kg respectively. The number of death in each group was recorded within a period of 72hrs. The lethal dose 50 was calculated using the following formula.

LD_{50} = √conc. with the highest death x lowest conc. without death.

Evaluation of Antioxidant Activity

DPPH radical scavenging activity

Evaluation of radical scavenging activities by antioxidants in the plant extract was carried out using DPPH (1, 1-diphenyl-2-picrylhydrazyl) radicals [9].

Different volumes of 0.2 mg/ml of the plant extract were added to 200 µl of (0.36 mg/ml concentration) DPPH solution in methanol. A series of concentration ranging from 2 to 15 µg of dried extract were tested. The mixtures were vigorously shaken and incubated in the dark for 30minutes after which the reduction of DPPH absorption was measured at 517 nm. Percentage inhibition by sample treatment was determined by comparing it with the methanol- treated control group. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

The IC50 values denote the concentration of each sample required to give 50 % of the optical density shown by the control, using a non-linear regression analysis. All test analysis were run in duplicates and both values were reported. Ascorbic acid (300 mg/ml) was used as the positive control.

Nitric Oxide Scavenging Activity

The nitric oxide scavenging activity was conducted based on the Greiss assay method (1858) which involves generating nitric oxide from sodium nitroprusside by the Greiss reaction.

2.0ml of 10mM sodium nitroprusside and 5.0ml of phosphate buffer were mixed with 0.5ml of plant extract and incubated at 25°C for 150 minutes. The samples were run as above but the blank was replaced with the same amount of water. After the incubation period, 2ml of the incubated sample was added to 2ml of Greiss reagent (1% sulphanilamide, 0.1% α-napthyl-ethydiaminedihydrochloride and 3% phosphoric acid) and then incubated for a period of 30 minutes. The absorbance of the pink chromophore formed by the diazotization of nitrite with α-napthyl-ethyl diaminedihydrochloride was measured at 540nm. Ascorbic acid was used as positive control. The experiment was performed in triplicate and the capacity to scavenge the nitric oxide was calculated using the following calculation

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Anti-lipid Peroxidation Activity

The effect of extract on lipid peroxidation inhibition was determined by the ammonium thiocyanate method. The principle is based on the measure of the absorbance of red colour at 500 nm which decreases in the presence of antioxidants. Different concentration (0.2-6 mg/ml) of the extract (0.5 ml) were mixed with 0.2 ml of diluted linoleic acid (25 mg/ml in 99 % ethanol) and 0.4 ml of 50 mm phosphate buffer (pH 7.4). After 15 minutes of incubation at 40 °C, an aliquot (0.1 ml) from the reaction mixture was mixed with reaction solution containing 3 ml of 70 % ethanol, 0.1 mL of ammonium thiocyanate (30 mg/ml in distilled water) and 50 µL of ferrous chloride (2.45 mg/ml in 3.5 % hydrochloric acid). The final reaction solution was mixed and incubated at room temperature for 3 minutes. The absorbance was then measured at 500 nm. Linoleic acid emulsion without extract served as control and vitamin C (0.2- 1 mg/ml) was used as standard control. Inhibition of linoleic acid oxidation was calculated by using the following formula:

$$\text{Anti-lipid peroxidation activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

IC50 values denote the concentration of sample which is required to inhibit 50% of Linoleic acid oxidation.

Statistical Analysis

The data obtained were subjected to statistical analysis using one way ANOVA to confirm their degree of significance at $p<0.05$ (95%) confidence level.

RESULTS

Acute toxicity and lethality test result

The acute toxicity study of the ethanolic extract of *synedrella nodiflora* leaves in mice gave an LD_{50} (lethal dose) value of the following below;

Table 1:Result of acute toxicity test

Doses	Number of animals per group	Number of mortality within 24 hours
8000mg/kg	2	2
7000mg/kg	2	2
6000mg/kg	2	0
5000mg/kg	2	0
4000mg/kg	2	0
3000mg/kg	2	0
2000mg/kg	2	0
1000mg/kg	2	0

The $LD_{50}$is thus calculated as= 7000× 6000 =42,000,000

Square root of √42,000,000 = 6480

Therefore, the LD_{50} is > 6400

Antioxidant Activity

Free radical scavenging activity of extracts of *Synedrella nodiflora* was investigated. They are represented as triplicate means±SD and shown below:

Table 2 The result of DPPH scavenging activity of the ethanolic extract of *Synedrellanodiflora*at different concentrations.

Table 2: DDPH scavenging activity

Concentration of extract	DDPH (%)	IC_{50} of DDPH
200 mg/ml	84.3±4.46[b]	
100 mg/ml	72.8±2.88[c]	
50mg/ml	58.63±2.99[d]	3.96mg/ml
250mg/ml	32.43±4.07[e]	
12.5 mg/ml	9.87±5.25[f]	
Ascorbic acid	97.53±1.03[a]	

Values are represented as mean± S.D for triplicate data. The values do not have the same subscripts and therefore shows significant difference ($p \leq 0.05$).

Table 3: Nitric oxide scavenging activity

Concentration of extract	Nitric oxide (%)	IC_{50} of Nitric oxide
200 mg/ml	70.70±3.52[b]	
100 mg/ml	58.36±3.38[c]	
50 mg/ml	39.27±2.29[d]	4.15mg/ml
250 mg/ml	26.20±2.16[e]	
12.5 mg/ml	19.90±7.13[e]	
Ascorbic acid	98.13±0.25[a]	

Values are represented as mean± S.D for triplicate data. Some of the values have the same subscripts while some do not. Those with the same subscript show no significant difference while those with different subscript show significant difference ($p \leq 0.05$).

Table 4: Anti-lipid peroxidation activity

Concentration of extract	Anti- lipid peroxidation (%)	IC_{50} of Anti- lipid
200 mg/ml	75.13± 4.80[b]	
100 mg/ml	53.97±5.08[c]	
50 mg/ml	36.37±3.99[d]	4.38mg/ml
250 mg/ml	28.04±5.59[d]	
12.5 mg/ml	8.13±7.13[e]	
Ascorbic acid	99.03±0.23[a]	

Values are represented as mean± S.D for triplicate data. Some of the values have the same subscripts while some do not. Those with the same subscript show no significant difference while those with different subscript show significant difference ($p \leq 0.05$).

Antimicrobial Test Results

Table 5: Result of minimum inhibitory concentration (MIC) of the plant extract.

Test organism	2000mg	1000mg	500mg	250mg	125mg	62.5mg	MIC
Escherichia coli	29.00±1.00	16.00±1.00	11.00±1.00	9.00±1.00	0.50±0.10	0.00±0.0	<125
Proteus mirabilis	41.00±1.00	33.00±1.00	16.00±1.00	0.00±.00	0.00±.00	0.00±.00	250
P. aeruginosa	11.00±1.00	5.00±1.00	3.00±1.00	0.00±.00	0.00±.00	0.00±.00	<500
S. aureus	31.00±1.00	23.00±1.00	17.00±1.00	12.00±1.00	6.00±1.00	0.00±.00	62.5
Salmonella typi	22.00±1.00	11.00±1.00	7.00±1.00	5.00±1.00	1.00±1.00	0.00±.00	<125
K. pneumoniae	27.00±1.00	18.00±1.00	9.00±1.00	7.00±1.00	0.00±.00	0.00±.00	125

Table 5 The result from the table above show the mean values of plant extract with their standard deviations (mean± SD) of minimum inhibitory concentration of the plant extracts of *synedrella nodiflora* on the six tested microorganisms.

Table 6: Result of antimicrobial activity of the plant extract and standard drug

Test organism	zone of inhibition for plant extract at 2000mg.kg	zone of inhibition forstandard drug (500mg)
Escherichia coli	29.00±1.00	43.00±1.00
Proteus mirabilis	41.00±1.00	46.00±1.00
P. aeruginosa	11.00±1.00	23.00±1.00
S. aureus	31.00±1.00	43.00±1.00
Salmonella typi	22.00±1.00	31.00±1.00
K. pneumonia	27.00±1.00	39.00±1.00

Table 6 the result from this table shows the antimicrobial activity of ethanolic extract of the leave of *synedrella nodiflora*, and a standard antibiotic (ciprofloxacin) which was used as a positive control and its antimicrobial activity was compared with that of leave extract of *synedrella nodiflora*. The values from the table show that standard drug has a higher zone of inhibition on *Proteus mirabilis* when compared with its plants extract.

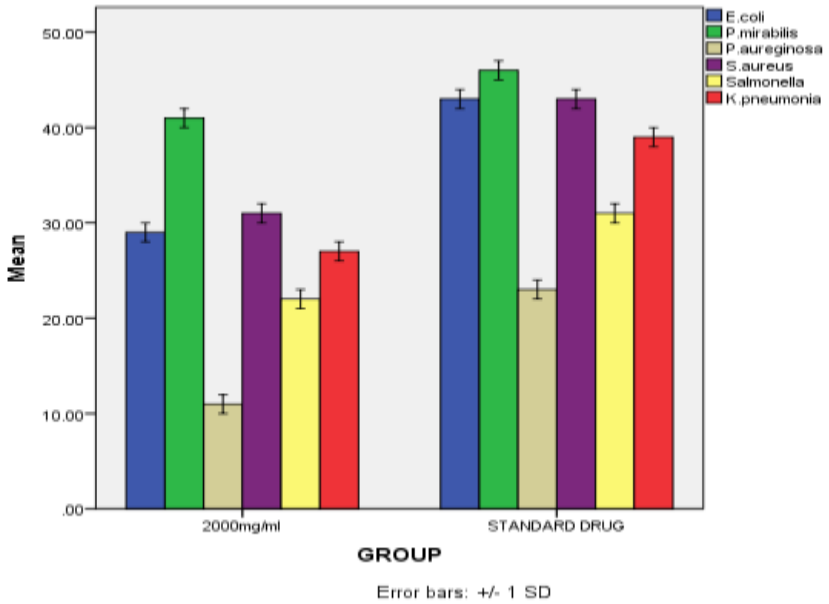

Fig-2: Bar chart showing the antimicrobial activity of the zone of inhibition of ethanolic leave extract of *synedrella nodiflora* (2000mg) and standard drug (ciprofloxacin 500mg).

There is a significant increase in the zone of inhibition on *proteus mirabilis* in the standard drug with a mean value of 46.00 which show the highest zone of inhibition, there is also a slight difference in the zone of inhibition on *proteus mirabilis* of the plant extract with a mean value of 41.00 when compared with its standard drug.

Table 7: Result on laxative activity

Groups	Faecal frequency for 24hours
2000mg	1.4500 ± .07071[a]
1000mg	1.8000 ± .56569[a]
500mg	1.0500 ± .21213[b]
250mg	1.7500 ± .07071[a]
125mg	1.1500 ± .35355[a]
62.5mg	1.1500 ± .07071[a]
Ducox (standard drug)	1.6000 ± .28284[a]
Normal saline	0.9500 ±.07071 [b]

The value from the table above shows that there was a significant ($p<0.05$) dose dependent increase in the faecal output of the rats when compared to the control group. The treatment group with the dose of 1000mg has the highest faecal output of 1.8000± 0.56569 followed by the treatment group with the dose of 250mg which have the faecal output of 1.7500± 0.07071. This implies that there was no significant difference between the extract and the control group. The effect of *synedrella nodiflora* increases significantly in the faecal output of rats compared to the control group and it shows a similar effect to that of the standard drug.

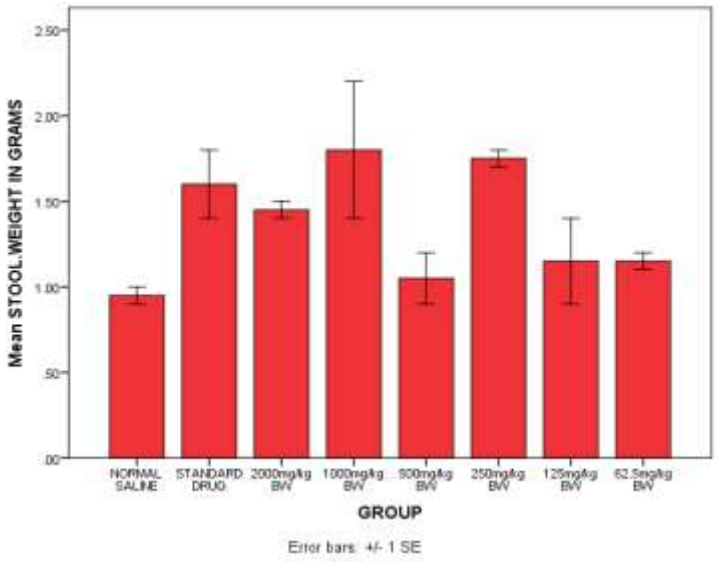

Fig-3: Bar chart showing the mean stool weight and groups of the experimental animals in grams

The above bar chart shows that at 1000mg of the treatment group the mean stool weight of the animal shows the highest faecal output than the standard drug (Ducox).

DISCUSSION

The laxative ability of medicinal plant could be as a result of stimulation of muscles in the walls of the small intestine and promotion of evacuation of the colon to generate an increased bowel movement [10]. Intake of sufficient amount of dietary fiber is a cornerstone in the prevention and treatment of constipation [11]. The results indicates that the plant *Synedrella nodiflora* has the ability to promote intestinal motility, stimulate intestinal transit as well as increase stool frequency and weight. There was a significantly ($p<0.05$) dose dependent increase in the faecal output of the rats when compared to the control group. This finding corroborates with the findings of earlier workers who had observed significant laxatives in experimental animals administered ethanol extract of *Marcya micrantha* [12] and ethanol extract of leaf and roots from *Amaranthus viridis* [10].

Infection diseases are the major cause of morbidity and mortality worldwide. The number of multi-drug resistance microbial strains and the appearance of strains which reduce susceptibility to antibiotics are continuously increasing. Such increase has been attributed to indiscriminate use of broad spectrum antibiotics and immunosuppressive agents. This situation provides the impetus to search for new antimicrobial substances from various sources like medicinal plants. The plants have traditionally provided a source of hope for novel drug compound. Plants herbal mixtures have made large contributions to human health and well-being. The use of plant extract with known antimicrobial properties can be of great significance for the therapeutic treatment. The ethanolic extract of *synedrella nodiflora* exhibited inhibitory zone against all the tested organisms.

From the result of this study, it was observed that *Proteus mirabilis* has the highest zone of inhibition for both the antibiotics and leave extract compared to other leave extracts.

It was also indicated that the ethanol extract of *synedrellanodiflora* leave have antibacterial activity on the entire tested organism but it has little effect on *pseudomonas aeruginosa*.

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular disease, inflammatory conditions, cancer and ageing [13]. Antioxidant may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent diseases. The percentage (%) inhibition scavenging activities of DDPH, Nitric oxide and Anti-lipid peroxidation showed a linear increase with increase concentration of the plant extract.

From the present study, It was showed that the control (vitamin c) has a high inhibitory scavenging activity of 97.53±1.03 and 200mg of DDPH has the highest inhibitory scavenging activity of 84.3±4.46 when compared to other concentration. This indicates that there is dose dependent relationship between the plant extract and the percentage scavenging activity. Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. However excess production of nitric oxide is associated with several diseases [14]. The nitric oxide scavenging activity has the highest inhibitory concentration of 98.13±0.25 for the control and The

plant showed a significant ($p<0.05$) dose dependent inhibitory potential.

The lipid peroxidation of membrane lipids initiated by oxygen radicals may lead to cell injury. Initiation of lipid peroxidation of ferrous sulphate takes place through OH radicals by Fenton reaction [15], thereby initiating a cascade of oxidative reactions. The results obtained shows that the control (vitamin c 100mg/ml) has the highest inhibitory scavenging activity of 99.03±0.23 and 200mg of the plant extract has the inhibitory scavenging activity of 75.13±4.80 which is higher when compared to other concentrations. This also indicates that there is dose dependent relationship between the plant extract and the percentage anti-lipid peroxidation activity.

CONCLUSION

The result from the study indicated that the activity of the plant extracts of *synedrella nodiflora* as antioxidant, antimicrobial and laxative agent were all concentration dependent.

REFERENCES

1. Meyer JJM, Afolayan AJ. Antibacterial activity of Helichrysum aureonitins species. Journal of Ethnopharmacology. 1997; 49:109-111.
2. Meyer JJM, Afolayan AJ, Taylor MB, Engelbrecht L. Inhibition of herpes simplex 1 by aqueous extracts from shoots of Helichrysum aureonites (Asteraceae) Journal of Ethnopharmacology. 1995; 51:41-43
3. Sule WF, Okonko IO, Joseph TA, Ojezele MO, Nwanze JC, Alli JA, Adewale OG, Ojezele OJ. In-vitro antifungal activity of Senna Alata Linn. Crude leaf extract. Advances in Applied Science Research. 2010; 1 (2): 14-26.
4. Wang J, Li J, Cao J, Jiang W. Antifungal activities of neem (Azadirachta indica) seed kernel extracts on postharvest diseases in fruits. Afr. J. Microbiol. Res. 2010; 4(11):1100-1104
5. Mshana NR, Abbiw DK, Addae-Mensah I, Adjanohoun E, Ahyi MRA, Enow-Orock. Traditional Medicine and Pharmacopoeia. Contribution to the revision of ethnobotanical and floristic studies in Ghana. Scientific, Technical and Research Commission of the Organization of African Unity, 2000.
6. Idu M, Onyibe HI. Medicinal Plants of Edo State, Nigeria. Res J Med Plant. 2007; 1: 32-41.
7. Amoateng P, Woode E, Kombian SB. Anticonvulsant and related neuropharmaco-logical effects of the whole plant extract of Synedrellanodiflora (L.) Gaertn (Asteraceae). J Pharm BioallSci. 2012; 4:140-8.
8. Woode E, Amoateng P, Abotsi WMK. Ethopharmacological analysis of the effects of the whole plant extract of Synedrella nodiflora (L.) Gaertn (Asteraceae) in murine models. Der Pharmacia Sinica. 2011; 2:54-67.
9. Omodamiro OD, Jimoh MA, Ajah O. Evaluation of the Anti-Inflammatory, Antimicrobial And Antioxidant Properties Of Aqueous Crude Gel Extract Of Aloe Buettneri. World Journal Of Pharmacy and Pharmaceutical Sciences. 2017; 6(8):124-134
10. Omodamiro OD, Obinna A. Laxative and antimicrobial activities of ethanolic extract of leaf and roots from Amaranthusviridis on wistar albino rats. Journal of Medicinal Botany. 2017; 1:42-46
11. Mostafa MH, Sahab AF, Elewa IS, Ziedan EH. Sesame transplanting strategies for controlling soil borne diseases. Egypt. J. Agric. Res. Nat. Res. Centre. 2003; 1 (2): 387–401
12. Takaharus, fujie, M, Yuji L, Kouji M, Hidiok. laxative and antidiarrheal activity of polycarbophil in mice and rats.J Pharmacol. 2002; 89;133-141.
13. Cross CE. Oxygen radicals and human diseases, Annual int. Med. 1987; 107-526.
14. Ialenti A, Moncada S, Di Rosa M. Modulation of adjuvant arthritis by the L-arginine: NO pathway. Br. J. Pharmacol. 1993; 108, lOP.
15. Halliwell B. Superoxide dependent formation of hydroxyl free radicals in the presence of iron chelates, FEBS let. 1978; 92: 321.