IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 15, Issue 5 Ser. III (Sep. –Oct. 2020), PP 58-65
www.Iosrjournals.Org

Anti-inflammatory, antimicrobial activities and phytochemical analysis of the tuber extracts of *Dioscorea oppositifolia* Linn.

[1]M. Sandhya Rani and [2]R.R.Venkata Raju*
[1]*Research* Scholar *Department of Botany, Sri Krishnadevaraya University, Anantapur- 515003, India*
[2]*Professor, Department of Botany, Sri Krishnadevaraya University, Anantapur- 515003, India*

ABSTRACT
Background: *The present paper deals with the phytochemical analysis, in-vitro anti-microbial activity and anti –inflammatory activity of tuber extracts of Dioscorea oppositifolia. The anti- inflammatory activity of the tuber extracts were subjected wistar albino rats in order to find out the pharmacological basis for its ethno-medicinal claims by adivasis properties.*
Materials and methods*: Acute toxicity studies were performed and produced no mortality in dose up to 5000 mg / b.wt and further screened for anti-inflammatory activity in carrageenan induced rat hind paw oedema.. The in-vitro antimicrobial activity was depicted by disk diffusion method using ethyl acetate, methanol and aqueous extracts.*
Results. *Preliminary phytochemical screening revealed the presence of alkaloids, phenols, flavonoids, steroids, tannins, glycosides, etc. All the test extracts exhibited significant antimicrobial activity on certain pathogens when compared to positive control. The results revealed that aqueous and methanol extracts were significantly effective at 100 mg / kg.b.w with 57.89; and 63.15% of inhibition respectively when compared with that of standard drug diclofenac*
Conclusion*: The data suggest that the tuber extracts of D.oppositifoia produce significant antimicrobial and anti-inflammatory principles that could be due to the effect of one or more bio active components in test extracts.*
Keywords: *Dioscorea oppositifolia, Phytochemical analysis, anti-inflammatory activity, carrageenan, wistar albino rats, antimicrobial activity.*

Date of Submission: 11-09-2020 Date of Acceptance: 26-09-2020

I. Introduction

Plants are the great source for the invention of pharmaceutical compounds and medicines. Natural products could be potential drugs for humans or live stock species and also these products and their analogues can act as intermediates for synthesis of useful drugs [1]. Plants possess many phytochemicals with various bioactivities including antioxidant, anti-inflammatory and anticancer [2] anti microbial activity [3]and one among such plants is *Dioscorea oppositifolia* , climber widely used in the treatment of many human and veterinary ailments in herbal and folk medicine. Inflammation is a normal protective response to tissue injury and it involves a posh array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair[4], which are aimed toward host defense and typically activated in most disease conditions. The critical role of inappropriate inflammation is becoming accepted in many diseases that affect man, including cardiovascular diseases, inflammatory and autoimmune disorders, neurodegenerative conditions, infection and cancer. Inflammation is component of the complex biological response of plant tissue to harmful stimuli such as pathogens, damaged cells, or irritant. Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Anti inflammatory drugs structure about half of analgesics, remedying pain by reducing inflammation as against to oploids, which affect the central nervous system.

Dioscorea oppositifolia L. belongs to the family Dioscoreaceae. The tuber is used for post pregnancy tonic [5] , anti diabetic [6] , swellings, scorpion stings and snake bites [7] and for toothache by local adivasis. The leaves, flowers, tender shoots and tubers are used in the form of decoction for leprosy and cancerous lesions for cooling and demulcent. The leaves are used for increase of sperm count [8],antiseptic for ulcers and abscesses. The whole plant is used in application for oedematous tumours and the ash extract of flowering twigs along with tender leaves to cure cancer and leprosy [9]. Previous reports indicate that contain phytochemicals such as Dioscorine [10] and Diosgenin [11] have been isolated from various species of *Dioscorea*. Pharmacological investigations have demonstrated that *Dioscorea* possess anthelmintic activity [11] , antioxidant activity [12], anti-inflammatory activity [13] and anti tumour activity [14], anti-diarrhoeal [15], antipyretic [16] analgesic [17] and anti microbial [18]. Due to its high value of medicinal usage, the present study was carried out

to evaluate the phytochemical composition, Antimicrobial activity and Anti-inflammatory activity of different solvents extracts of the tubers of *D.oppositifolia*.

II. Materials And Methods:

Collection and identification of plant Material :

Plant material was collected from Tirumala and Talakona hills along the Seshachalam Hill Ranges of Eastern Ghats of Andhra Pradesh during August-November, 2017 and its identification was authenticated using flora of Kurnool (Venkataraju and Pullaiah) and also compared with that of MH, Coimbatore and voucher specimens (No:40352) were preserved in the herbarium (SKU), Department of Botany, S.K.University, Anantapur as per the standard method [19]. Tubers were thoroughly washed, cut in to pieces and further dried under shade at 28 ± 2 º C for about 10 days. The dried parts were ground well in to a fine powder in a mixer grinder and sieved to particle size of 50 – 150mm. The powders were stored in a polythene bags at room temperatures.

Extract preparation:

Shade dried tuber powder was subjected to soxhlet extraction with n-hexane, ethyl acetate and methanol. Simultaneously aqueous extract also prepared, and all samples reduced to semisolid extracts and the same were preserved in air tight bottles at 4^0C in a refrigerator until further use.

Chemicals and instruments:

All chemicals used in the estimation were of analytical grade. Carrageenan was purchased from sigma chemicals; reference standard Diclofenac sodium was obtained from Apollo Pharmaceuticals, Anantapur, India. Measuring the oedema sliding Vernier calipers was used.

Microorganisms used:

The microbial strains viz., , *Micrococcus luteus* MTTC 2470, *Klebsiella pneumoniae* MTCC 7028, *Pseudomonas aeruginosa* MTCC 7296, *Salmonella enterica* MTCC 98, *Fusarium oxysporum* MTCC1272 and *Candida albicans* MTTC 854, were used to test with different extracts. The standard micro-organisms were obtained from the Microbial Type Culture Collection Centre, Institute of Microbial Technology (IMTECH), Chandigarh, India.

Animals:

Adult male Wistar albino rats (150g -180 g) for the *in-vivo* evaluation of anti-inflammation activity was purchased from Sri Venkateswara Traders, Bengaluru. They were housed under standard laboratory conditions and were fed with standard animal feed *ad libitum* and water. The experimental protocol was approved by institutional animal ethical committee No: (Protocal No .SKU/Biochem /03/2016)

Phytochemical analysis:

The n-hexane, ethyl acetate, methanol and aqueous tuber extracts of *D.oppositifolia* were qualitatively analysed to find out the phytoconstituents as per standard methods (21; 22; 23; 24; 25 ;26).

Antimicrobial activity:

The antimicrobial activity of the extracts was evaluated by disc diffusion method [27][28]. Previously prepared paper discs containing different concentrations of solvent extracts were placed on the surface of the petriplates, containing 20 ml of respective media seeded with 0.1 ml of previously prepared microbial suspensions (10^5CFU/ mL).The discs containg methanol, ethyl acetate and aqueous extracts used as control . Ciproflaxin (10 µg/disc) used as positive control. The discs containing ethyl acetate , methanol and water served as negative controls. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. The plates were incubated for 24 h at 37°C and the diameter of the inhibition zones was recorded. Three independent trials were conducted for each concentration.

Acute toxicity studies:

Acute oral toxicity study was performed as per OECD-423 guidelines and Wistar rats (n =6) were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level of 1000, 1500, 2000, 2500, 3000, 5000 mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. Mortality was observed and then the LD50 was calculated.

Anti-inflammatory study:

Male Wistar rats were used as the animal model for acute inflammation as per the standard methods [29] . Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of all experimental rats 1h after the oral administration of test materials. The paw volume was measured by dorso ventral measurements of rat hind footpad (paw diameter) using a sliding vernier calipers before and at 0.5, 1, 2, 3, 4 and 5 hr intervals after the carrageenan injection. The

tuber extract was administered at 100, 300, 500 and 1000 mg/kg b.w. Diclofenac sodium 100 mg/kg b.w was used as standard anti-inflammatory drug. Percentage of inhibition of oedema volume between treated and control was calculated as follows:

$$\% \text{ inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc = Mean increase in paw volume of the control group. Vt = Mean increase in paw volume of treated group.

Nine groups of six rats each were taken. Group I treated with normal 2% Gum acacia solution of 1ml/kg b.wt, Group II received carrageenan; Group III treated with Diclofenac by oral route; Group IV- IX receive tuber aqueous and methanol extracts at a concentrations of 250, 500 and 1000mg respectively along with 1% carrageenan 0.1ml and 2% Gum acacia Solution.

Statistical Analysis: All values were expressed as Mean ± SEM, and data was analyzed by one way analysis of variance (ANOVA) followed by Dunnett's -test using Graph Pad Instat.

III. Results:

Preliminary phytochemical analysis:

Phytochemical screening of the various extracts of tubers of *D. oppositifolia* showed the presence of alkaloids, flavonoids, triterpenoids, phenols, tannins, saponins, steroids, quinines, carbohydrates, protein, amino acids, lignins, Polyoses etc. However, some phytoconstituents were absent in some extracts as reported (Table 1). This variation in the results could be due to the difference in the polarity of solvents used for extraction. The phytoconstituents, gums and mucilage, carotenoids, emodins and coumarins were not reported in any of the plant extract. All the extracts were reported to exhibit positive result in color reaction only for alkaloids, flavonoids and phenols. The phytochemical constituents of the plant extracts investigated are summarized in Table 1.

Table 1: Qualitative analysis of the phytochemicals present in the tubers of *D. oppositifolia* Linn.

Phytochemicals	n-Hexane	Ethyl acetate	Methanol	Water
Saponins	-	-	+	+
Flavonoids	+	+	+	+
Triterpenoids	-	-	+	+
Alkaloids	+	+	+	+
Glycosides	-	-	+	+
Gums and Mucilage	-	-	-	-
Steroids	-	+	+	+
Carbohydrates	-	-	+	+
Protein and Amino acids	-	-	-	+
Tannins	-	-	+	+
Phenolic compounds	+	+	+	+
Anthocyanins and Anthocyanidins	-	-	+	+
Carotenoids	-	-	-	-
Coumarins	-	-	-	
Emodins	-	-	-	-
Lignins	-	-	++	-
Polyoses	-	-	+	+

(+) Positive (-)Negative

Antimicrobial activity: The antimicrobial activity of *D.oppositifolia* solvent (methanol, ethyl acetate and aqueous) extracts was studied at different concentrations (0.25,0.50 and 1 mg/ml) against human pathogenic micro organisms :Gram positive and Gram negative bacteria and fungal strains are presented in table 2.The methanol extract showed the potent inhibitory effect against *S.enterica* 20mm compare to positive control. The ethyl acetate extract exhibited highest inhibition zone against *C.albicans* (20mm) whereas least against *M.luteus* (13mm).The methanol and ethyl acetate extracts showed maximum antimicrobial activity while aqueous extract exhibited minimum against all test micro organisms with zone of inhibition range of 8-17mm. At 1mg/ml, inhibition zone of solvent extracts were more than 0.25 and 0.5mg/ml. The antimicrobial activity of different solvent extracts exhibited concentration dependant performance.

Table:2 Anti microbial activity of different solvent extracts of tubers of *D.oppositifolia* / (solvent extracts of *D.oppositifolia* of tubers)

S. N	Microbial strains	Solvent extracts (Zone of inhibition in mm^{-1})						
		Methanol		Ethyl acetate		Aqueous		Ciproflaxin
		0.50 mg/ml	1 mg/ml	0.50mg/ml	1mg/ml	0.50mg/ml	1mg/ml	1mg/ml
1	*Micrococcus luteus*	1.03±0.1	1.46±0.1	1±0.1	1.36±0.15	0.96 ±0.05	1.1±0.1	3.13±0.15
2	*Salmonella enterica*	1.5±0.1	2.03±0.1	1.1±0.2	1.63±0.05	1.1±0.1	2.73±0.63	4.03±0.15
3	*Klebsiella pneumoniae*	1.23±0.1	1.46±0.1	1.46±0.11	1.6±0.1	1.2±0.05	0.86±0.05	5.1±0.1
4	*Pseudomonas aeruginosa*	1.6±0.1	1.73±0.1	1.2±0.17	1.66±0.11	1.23±0.15	1.50±.52	3.93±0.05
5	*Fusarium oxysporum*	1.2±1.1	1.43±0.1	1.1±0.1	1.43±0.11	10. ±1	1.2±0.1	4.26±0.058
6	*Candida albicans*	1.5±1.6	1.96±0.1	1.3±0.1	2.06±0.05	1.73±0.11	1.66±0.15	4.3±0.2

Each value represents Mean ± SD of three replicates.

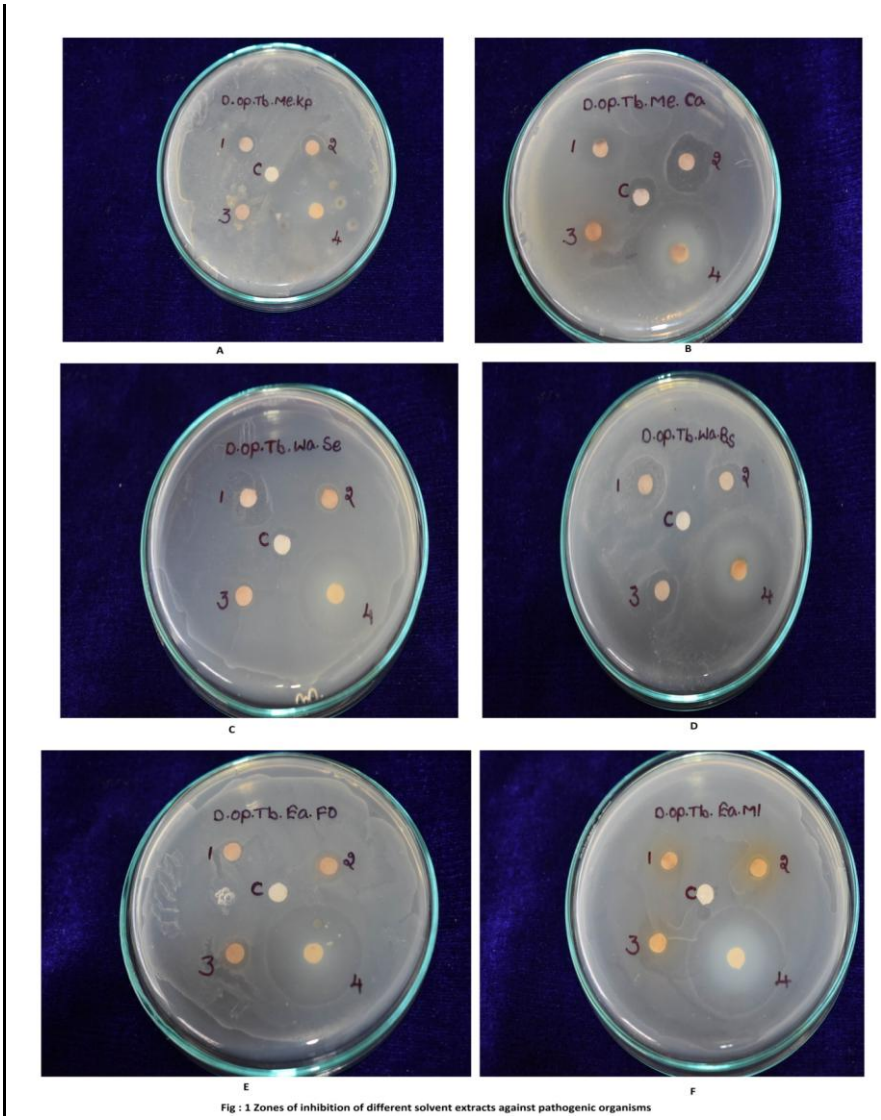

Fig : 1 Zones of inhibition of different solvent extracts against pathogenic organisms

Antimicrobia activity of different extracts of D.oppositifoia in three different concentations.
1)25mg/ml ; 2)50 mg/ml ;3)75 mg/ml ;4) Antibiotic and C= control against different strains.
A,B= Methanol; C,D =Ethyl acetate; E,F= Aqueous

Acute Toxicity Studies : Acute toxicity studies with Aqueous and methanol tuber extracts of *D.oppositifolia* in a single oral dose did not show any significant toxicity signs on Wistar albino rats when observed for first four hours and followed by daily observations upto 14 days and no mortality and also there is no signs and symptoms such as changes in body weight and food intake, psychomotor activities, restlessness, respiratory distress,

diarrhoea, convulsions and coma. And it was found safe up to the dose of 5000 mg/kg b.wt according to OECD guidelines 425. Hence 1/5th and 1/10th dose of 5000mg/kg (LD50) 1000mg and 500 mg/kg b.wt can be used as safe dose for experimental studies.

Tabel:3: Effect of Tuber Extracts on Carrageenan- Induced rat hind paw oedema (mm)

Extracts	Dose mg /kg. b.wt.	Diameter of Paw edema in mm						% of Inhibition
		0.5hr	1hr	2hr	3hr	4hr	5hr	
Normal		3.0±0.04	3.0±0.04	3.0±0.00	3.1±0.04	3.0±0.04	3.0±0.04	
Control		7.1±0.09	8.1±0.04	8.4±0.04	9.1±0.04	9.3±0.04	9.5±0.04	
Diclofenac	100	7.8±0.06	5.9±0.12	4.1±0.25	3.9±0.10	3.6±0.08	3.5±0.04	63.15%
Aqueous	250	7.3±0.15	6.6±0.00	5.8±0.12	5.3±0.15	4.4±0.06	4.0±0.00	57.89%
	500	6.7±0.06	6.1±0.12	5.4±0.17	4.8±0.10	4.0±0.12	3.5±0.03	63.15%
	1000	5.9±0.12	5.7±0.15	4.9±0.06	4.6±0.06	3.4±0.03	2.6±0.04	72.63%
Methanol	250	6.5±0.00	5.6±0.10	5.2±0.06	4.7±0.10	4.0±0.12	3.5±0.04	63.15%
	500	5.8±0.12	4.9±0.10	4.5±0.06	4.1±0.03	3.6±0.03	3.1±0.10	67.36%
	1000	5.1±0.10	4.6±0.06	4.4±0.15	3.9±0.10	2.9±0.05	2.0±0.05	78.94%

Anti-inflammatory Activity: Carrageenan induced rat paw oedema was reduced by the methanol extracts at 250 mg/kg b.wt more effectively than aqueous extracts compared to the standard drug Diclofenac at 100 mg/kg b.wt. The most effective activity observed with tuber methanol at 250 mg/kg b.wt showed equal activity to that of diclofenac with 63.15% of inhibition of inflammation (Table-3; Plate-1).

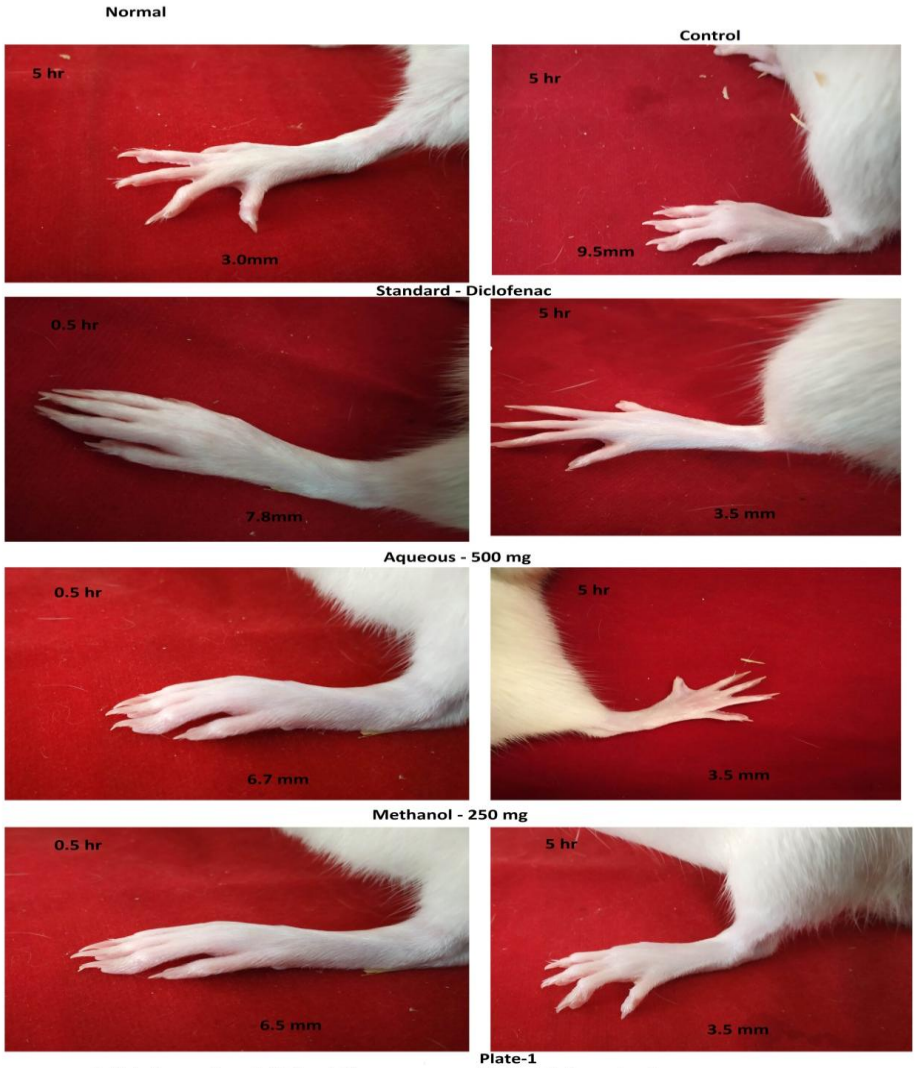

Plate-1

Anti- inflammatory Activity of *Dioscorea oppositifolia* L.tuber extracts

IV. Discussion:

Phytochemical constituents like alkaloids, flavonoids, tannins, phenols, saponins, and number of other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other herbivores[29]. The present study administered on the plant samples revealed the presence of medicinally active constituents. Martin'[30]reported a bioactive compound Diosgenin, is a sapogenin used in the synthesis of steroidal drugs. Several publications represents the highest ever estimated diosgenin content in plant material, measured by Behera *et al*.[31] in *D.zingiberens*is ,*D.pubera, D.spicata, D.hispida* and *D.*hamiltonii[32]. Poornima and Ravishankar,[33] repoerted saponins, alkaloids, flavonoids, tannins, and phenols in *D.belophylla*. Cardiac glycosides content found in methanol extract, have been used for over two centuries as stimulant in case of cardiac failure[34;35]. Further terpenes or terpenoids are active against bacteria [36;37;38]. The presence of terpenoids shows that it might be effective against any bacterial infections. Different types of phenolic compounds present in tubers and other parts of *Dioscorea* species [39;40;41;42].

According to previous reports *Dioscorea* species associate secondary metabolites which are proved to be very good antimicrobial agents. These bioactive compounds are known to act by different mechanism and exert antimicrobial action. Tannins bind to proline rich proteins and intrude with the protein synthesis[43]. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a good array of microorganisms. Their activity is possibly due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls[44]. Diosgenyl saponins, one of the most abundant steroid saponins, with diosgenin as the steroidal sapogenin, are reported to exert a large variety of biological functions such as antifungal, antibacterial, and anticancer[45]. Alkaloid is one among the phytochemical compounds identified during this study. It has been allied with medicinal uses for hundreds of years. Most common biological properties of alkaloids are their toxicity against cells of foreign organisms, anti-inflammatory, anti-asthmatic, and anti-anaphylactic properties[46;47;48] .The studies of Quan *et al*,[49] reported potent antibacterial activity against *Bacillus subtilis* and *S. aureus* of diosgenin derivatives like 2,6- iodo-pseudogiosgenin and 2,6 iodo-psuedogiosgenone. Sautour *et al*,[50]showed steroidal saponins from *D.cayenensis* to possess activity against *C. albicans*. Therefore, the presence of phytochemicals could justify the observed antifungal activities in the current study. The CH Cl -soluble portion of the crude extract and the two clerodanes showed significant activities against *P. aeruginosa, Salmonella typhi, S.paratyphi A and S.paratyphi B*, which was reported by Teponno *et al*[51]. Inflammation is a common phenomenon and it is a reaction of living tissues towards injury [52].The development of odema in the paw of the rat after the injection of carragenan is due to the release of histamine, serotonin, prostaglandin[53]. Most widely used anti-inflammatory drugs are non steroidal (NSAID). Long term usage of NSAID may induce gastro-intestinal ulcers, bleeding and renal disorders due to their non selective inhibition of cyclo oxygenate (COX-1 & COX-2) enzymes further leads for the disturbance of arachidonic acid metabolic pathway produces prostaglandins which causes pains and inflammation [54;55;56]. *D.oppositifolia* tuber methanol extracts showed effective anti inflammatory activity at 100 mg/kg b.w on rats proved up to 61.05% of inhibition. This is in accordance with the previous reports[14;28]. In the year 2007, Wantana Reanmongkol et al[57] observed the anti inflammatory effect with ethanol and aqueous exracts of tuber of *D.membranacea*. Panduranga et al.,[58] reported anti inflammatory activity of ethanolic leaf extract of *D.hispida*. *D.oppositifolia* tuber extracts possess phenols, diosgenin supports its anti inflammatory activity [31;33].The results of the present study indicates that extracts of tuber of *D.oppositifolia* possess significant anti-inflammatory activity on acute inflammation. Similar results were also obtained in the earlier reports of *Dioscorea* species [14;59].

In conclusion, *D.oppositifolia* contain potential anti- inflammatory and phytochemical components that may be of great use for the development of pharmaceutics as a therapy against various human and veterinary ailments. The plant crude extracts could serve as potential sources of new antimicrobial, anti-inflammatory agents. The presence of flavonoids, tannins and phenol may have contributed to the observed pharmacological activity, based on this, further attempts are being made to evaluate the therapeutic potance of biodynamic active principles present in the test species.

Acknowledgment:

Authors are thankful to the department of Botany, S.K.University, Anantapur, Andhra Pradesh, India for providing necessary facilities for competing the present study.

References:

[1]. Makkar HPS, Norvsambuu T, Lkhavatsere S, Becker K. 2009. Plant secondary metabolites in some medicinal plants of Mongolia used for enhancing animal health and production. Tropicultura 27 (3): 159-167.
[2]. Wu J and Yang B.B.Anticancer activity of Hemsya amabitis extract. Life sciences,2002,71,2161-2170.
[3]. Kaneria M, Kanari B, Chanda S. Assessment of effect of hydroalcoholic and decoction methods on extraction of antioxidants from selected Indian medicinal plants. Asian Pac J Trop Biomed, 2012; 2(3): 195-202.
[4]. Vane, J.R., Botting, R.M., 1995. New insight into themode of action of anti-inflammatory drugs. Inflamm Res. 44, 1-10.
[5]. Mishra S, Swain S, Chaudhary SS, Ray T. Wild edible tubers (Dioscorea spp.) and their contribution to the food security of tribes of Jaypore tract, Orissa, India. Plant Genet. Resour 2008; 156:63-67
[6]. Shali Saheb, B Ravi Prasad Rao, M Venkateswarlu and M Swamulu Medicinal plants used for jaundice by the tribal people of nallamalais in Andhra Pradesh Journal of Pharmacognosy and Phytochemistry 2018; 7(4): 528-531
[7]. Dutta B. (2015). Food and medicinal values of certain species of *Dioscorea* with special reference to Assam. J. Pharmacog. Phytochem. 3, 15–18.
[8]. Sharma LN, Bastakoti R. Ethnobotany of Dioscorea L. with emphasis on food value in Chepang communities in Dhading District, central Nepal. Botanica Orientalis. J. Plant Sci 2009; 6:12-17.
[9]. Felix R, Nirmal Kumar N and Leon Stephan Raj T, 2009. Pharmacognostical Study of *Dioscorea oppositifolia* L. *Ethnobot Leaflets.*, 13: 77-82.
[10]. Ayer, D.E., Büchi, G., Reynolds Warnhoff, P. and White, D.M. The Structure of Dioscorine. Journal of the American Chemical Society,1958, 80 (22), 6146.
[11]. Gao-Xue wang. Dong-Xin Jiang. JunLi. Jing Han Anthelmintic activity of Steroidal saponins from Dioscorea Zingiberensis C.H. Wright against Dactylogyrus intermedius(Monogenea) in gold fish(carassius auratus), Northwest A and F university , China.prasitol Res 2010, vol.107(6),1365-1371.
[12]. Sudawadee Theerasin and A.T.Baker Analysis and Identification of phenolic compounds in Dioscorea hispida Dennst. Chemistry Department, Phranakhan Si Ayutthaya Rajabhat University, Thailand Department of chemistry, Australia. As .J. Food Ag-Ind.2009, 2(04), 547-560.
[13]. Olayemi J.O. and Ajaiyeoba E. O. Antiinflammatory studies of yam (Dioscorea esculenta)extract on wistar rats Afr. J. Biotechnol 2007 Vol.6(16):1913-1915
[14]. Iuiyuan Gao I, Bailing I iou. Constituents from anti-tumor-promoting active part of Dioscorea bulbifera in JB6 Mouse epidermal cells. A school of traditional Chinese Materia medica. Shenyang pharmaceutical university, Shenyang, China. 2007, 2 (3) 104-109.
[15]. Jadhav VD, Mahadkar SD, Valvi SR. Documentation and ethnobotanical survey of wild edible plants from Kolhapur District. Rec. Res. Sci. Technol. 2011; 3:58-63
[16]. Singh N, Pangtey YPS, Khatoon S, AKS Rawat. Some ethnobotanical plants of Ranikhet region, Uttaranchal. J. Econ. Taxon. Bot. 2009; 33:198-204.
[17]. Mbiantcha M, A Kamanyi, Teponno RB, AL Tapondjou, P Watcho, TB Nguelefack. Analgesic and anti-inflammatory properties of extracts from the bulbils of Dioscorea bulbifera L. var sativa (Dioscoreaceae) in mice and rats. Evid-Based Compl. Alt. 2011, 912935.
[18]. Kokkaiah Irulandi, Sethupandian Geetha, Palanichamy Mehalingam Antioxidant, antimicrobial activities and phytochemical analysis of leaves extracts of Dioscorea wallichii Hook. f. Journal of Applied Pharmaceutical Science Vol. 6 (11), pp. 070-074, November, 2016.
[19]. Jain S.K, Rao R.R. A Handbook of Field and Herbarium Methods. Today and Tomorrow's Printers and Publishers, New Delhi, 1977.
[20]. Kokate CK. Practical Pharmacognosy, Vallabh Prakashan, Delhi 2007: 107-111.
[21]. Harbone JB. Phytochemical methods, Chapman and Hall, Ltd, London, 1973: 188.
[22]. Harbone JB. Phytochemical Methods, Chapman & Hall, London, 1999: 60-66
[23]. Edeoga HO, Okwu DE Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol, 2005; 4: 685-688.
[24]. Yadav M, Chatterji S, Gupta SK, Watal G. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. Int J Pharm Sci, 2014; 6(5): 539-542.
[25]. Gopinath SM, Suneetha TB. and Mruganka VD. Chemical prophylaxis and antibacterial activity of Methanolic and aqueous extracts of some medicinal Plants against bovinemastitis. International Journal of Advanced Biological Research, 2102; 1 (1): 93-95.
[26]. Bayer AW, Kirby MDK, Sherris JC, Truck M.1966.Antibiotic susceptibility testing by standard singe disc diffusion method. Am. J. Clinical Pathol.60:493-496
[27]. Cruickshnak, R., 1968. 11 ed. Medicinal microbiology: 6. Corine, D.D., D. Michel and J. Quetin-Leclercq, 2000. A guide to diagnosis and control of infection. Antimicrobial activity of bark extracts of Syzygium Edinburgh and London: E and S Livingston Ltd, jambos (L.) Alston (Myrtaceae). Journal of pp: 888.
[28]. Winter C.A, Risley E.A, Nuss G.W. Carrageenan- induced edema in hind paw of the rats as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Bio. Med. 1962; (111):544-547.
[29]. Bonjar GHS, Nik AK, Aghighi S. Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran. J. Biol Sci, 2004; 4:405-412
[30]. Martin F. W. (1969). The species of Dioscorea containing sapogenin, Economic Botany, 23(4): 373-379.
[31]. Behera K.K., sahoo S and Prusty A.(2010). Biochemical quantification of diosgenin and ascorbic acid from the tubers of different Dioscorea species found in Odisha, Libyan Agriculture Res.cent.J.Int., 1(2):123-127.(a)
[32]. Asha K. I., Nair G. M. (2005). Screening of *Dioscorea* species for diosgenin from southern western Ghats of India. Indian J. Plant Genet. Resour. 18, 227–230.
[33]. Poornima G. N. and Ravishankar R. V.(2007). In vitro propagation of wild yams, Dioscorea oppositifolia (Linn.) and Dioscorea pentaphylla(Linn.), African Journal of Biotechnology, 6(20): 2348-2352.
[34]. Olayinki AO, Onuruvwe O, Lot TY. Cardiovascular effects of the Methanolic extract of the stem bark of *Khaya sengaensis*. Phytotherapy Research 1992; 6(5):282-284.
[35]. Trease GE, Evans WC. Pharmacology. Edn 11,Braillieriere Tindall Ltd., London, 1998, 60-75.
[36]. Barre JT, Bowden FB, Coll JC, Jesus J, Fuente VE, Janairo GC *et al*. A bioactive triterpene from *Lantana camara*. Phytochemistry 1997; 45:321-324.
[37]. Habtemariam S, Gray AI, Waterman PG. A new antibacterial sesquiterpene from *Premaoligotricha*. Journal of Natural products 1993;56:140-143.

[38]. Scortichini M, Pia RM. Preliminary in vitroevaluation of the antimicrobial activity of terpenes and terpenoids towards Erwinia amylovora(Burkill) Winslow *et al*. Journal of Applied Bacteriology 1991; 71:109-112.
[39]. Sara C., Lucas L., Keast R. (2010). Biological activities of phenolic compounds present in virginolive oil. Int. J. Mol. Sci. 11, 458–479.10.3390/ijms11020458.
[40]. Liu H., Tim K. W., Chou G. X., Wang J. M., Ji L. L., Wang Z. T. (2011). Phenolic compounds from the rhizomes of *Dioscorea bulbifera*. Chem. Biodiver. 8, 2110–2116.10.1002/cbdv.201000279.
[41]. Aderiye B. I., Ogundana S. K., Adesanya S. A., Roberts M. F. (1996). Antifungal properties of Yam (*Dioscorea alata*) peel extract. Folia Microbiol. 41, 407–412. 10.1007/BF02815690.
[42]. Theersin S., Baker A. T. (2009). Analysis and identification of phenolic compounds in *Dioscorea hispida* Dennst. Asian J. Food .Agro .Indus. 2, 547–560.
[43]. Shimada T. Salivary proteins as a defense against dietary tannins. J. Chem. Ecol. 2006; 32 (6): 1149-1163.
[44]. Marjorie C. Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. 1999; 12: 564-582.
[45]. Li B, Yu B, Hui Y, Li M, Han X, Fung KP. Salt-assisted acid hydrolysis of starch to D- glucoseunder microwave irradiation. Carbohydr Res. 2001;331:1.
[46]. Gopalakrishnan C, Shankaranarayan D, Kameswaran L, Natarajan S. Pharmacological investigations of tylophorine, the major alkaloid of *Tylophora indica*.
[47]. Ganguly T, Sainis KB. Inhibition of cellular immune response by *Tylophora indica* In experimentalmodels. Phytomed. 2001;8(5):348–55. doi: 10.1078/0944-7113-00055.
[48]. Staerk D, Lykkeberg AK, Christensen J, Budnik BA, Abe F, Jaroszewki JW. In vitro Cytotoxicactivity of phenanthroindolizidine alkaloids from *Cynanchum vincetoxicum* and *Tylophora tanake*against drug-sensitive and multidrug-resistant cancer cells. J of Natural Prod. 2002;65(9):1299–302.doi: 10.1021/np0106384.
[49]. Quan HJ, Koyanagi J, Hagiwara K, Cui XR, Isshiki Y, Kondo S, et al. Reactions of 26-iodopseudodiosgenin and 26-iodopseudodiosgenone with various nucleophiles and pharmacological activities of the products. Chem Pharm Bull. 2006;54:72–9.
[50]. Sautour M, Mitaine AC, Miyamoto T, Dongmo A, Lacaille-Dubois MA. A new steroidal saponins from *Dioscorea cayenensis*. Chem Pharm Bull. 2004;52:1353–5.
[51]. Teponno RB, Tapondjou AL, Gatsing D, Djoukeng JD, Mansour E, Tabacchi R, et al. Bafoudiosbulbins A, and B, two anti-salmonellal clerodane diterpenoids from *Dioscorea bulbifera L* var sativa. Phytochem. 2006;67:1957–63.
[52]. Mohamed STK, azeem AK, Dilip C,Sankar C, Prasanth NV, Duraisami R. Anti inflammatory activity of the leaf extracts of Gendarussa vulgaris Ness. Asian Pac J Trop Biomed 2011:4(2;147-149).
[53]. George will OA, Georgewill UO, Nwankwoala RNP. Antiinflammatory effects of Moringa oliefera lam extracts in rats. Asian Pac J Trop Biomed 2010;3(2):133-135.
[54]. Robert. A. Antisecretory, antiulcer, cytoprotective and diarrheogenic properties of prostaglandins. Advances in Prostagladins and Thrombaxane Research. 1976; 2; 507-520.
[55]. Peskar. B.M. On the synthesis of prostagladins by human gastric mucosa and its modification by drugs. Biochimica et Biophysica Acta. 1977; 2; 307-314.
[56]. Tapiero. H, Nguyen Ba, Couvreur, Tew. K.D. Poly unsaturated Fatty Acids (PUFA) and eicosanoids in human health and pathologies. Biomedicine and Pharmacotherapy. 2002; 56 (5); 215-222.
[57]. Wantana Reanmongkol1 , Arunporn Itharat2 and Pisit Bouking3Investigation of the anti-inflammatory, analgesic and antipyretic activities of the extracts from the rhizome of Dioscorea membranacea Pierre in experimental animals Songklanakarin J. Sci. Technol. Vol.29 (Suppl. 1), March 2007 :
[58]. Panduranga M. G., Punith K. T. G., Suresh A., Ravishankar H. G., Chandrasekher K. B. And Lokesh S. (2011). Evaluation of ethanolic leaf extract of Dioscorea hispida Dennst. for anti-inflammatory and analgesic effect, International Journal of Pharmacy & Industr Indian J Med Res. 1979;69:513–
[59]. Omodamiro O.D. Anti-inflammatory and Diuretic Activities of Ethanol Extract of Dioscorea bulbifera Leaf American Journal of Drug Delivery and Therapeutics 2015(2][1]:029-038.

M. Sandhya Rani, et. al. "Anti-inflammatory, antimicrobial activities and phytochemical analysis of the tuber extracts of Dioscorea oppositifolia Linn." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 15(5), (2020): pp. 58-65.