Journal of Sustainable Veterinary & Allied Sciences
P-ISSN-2695-2661 e-ISSN: 2811-1346
JoSVAS 2022, Vol 3, Issue 1: 1-7
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Original Research Article

Comparative anti-inflammatory and analgesic effects of fractions of *Allium cepa* l. bulb red cultivar extracts in rats and mice

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ABSTRACT

Pain and inflammation are amongst the most classical clinical signs of acute and chronic common diseases in humans and animals. Globally, these had led to losses of human and financial resources. This was designed to compare the anti-inflammatory and analgesic effects of four fractions of serially produced extracts of red cultivar *Allium cepa* L. bulbs in laboratory rats and mice. Extracts of red cultivar *Allium cepa* L. bulbs were serially extracted by macerating oven dried onion bulbs in four (4) solvents (N-Hexane, Ethyl acetate, Chloroform and methanol). The anti-inflammatory and analgesic tests were carried out using Carrageenan induced paw oedema in rats and acetic acid induced abdominal writhe in mice respectively. The results revealed that while the extracts of solvents on either extremes of the polarity index (n-Hexane and methanol) were very mild in their anti-inflammatory effects, the effects of chloroform and ethyl acetate were 74.36% and 78.85% inhibition, respectively. However, the analgesic effects of all the extracts were generally low. The 50 mg/kg methanol fraction of the red cultivar *A. cepa* was significantly better than all the other fractions with 35% abdominal writhe inhibition in mice. This research shows that ethyl acetate fraction of the red cultivar *Allium cepa* extracts performed excellently as an anti-inflammatory while none of the extracts exhibited appreciable analgesic effect.

Key word: Analgesic; anti-inflammatory; solvent polarity; red cultivar *Allium cepa* L

INTRODUCTION

Pain and inflammation are of great constitute great challenges world-wide been considered to be a major social, clinical, and economic problem around the world. Pain and inflammatory disorders are linked to wide range of undesirable effects that culminate into poor physical and economic soundness or even death. (Vietri *et al*., 2015; Kawai *et al*., 2017). Medicinal plant uses are common indigenous and modern practitioners for therapeutic and complementary medical purposes. Medicinal plants naturally possess extractable secondary metabolites such as flavonoids, tannins, glycosides, alkaloids and saponins (Oyewusi *et al.,* 2015). These bioactive metabolites confer therapeutic properties for the treatment of many diseases such as antihypertensive, antibacterial, anti-inflammatory, antioxidant (De-Monte *et al.,* 2014; Zlotek *et al.,* 2016).

Extraction of bioactive compounds from medicinal plants provides a whole lot of opportunities for production of new therapeutic agents (Sasidharan *et al.,* 2011). Extraction of bioactive principles from medicinal plant material is necessary to obtain the targeted bioactive compounds from plant materials which can be presented as potential drug candidates for specific diseases when refined and purified. Bioactive compounds are isolated for pharmacological assessment, identification and characterization for production of new drugs (Paul *et al.,* 2015).

These bioactive (phytochemicals) compounds can be obtained from plants through several ways which include homogenization, milling, grinding and extraction. Solvent extraction is the most commonly used of these preliminary methods of screening potential remedies.

Bioactivity guided extraction of compounds or substances require the use of specific bioactivity models to ensure the compounds of interest are perpetuated in the process of compound separation. Serial exhaustive compound extraction procedure involves successive application of solvents of increasing polarity from a non-polar (low polar) solvent such as N-hexane (Ayaffor *et al.,* 1994) to medium

http://doi.org/10.54328/covm.josvas.2022.081

polarity such as ethyl acetate and to highly polar solvents such as ethanol, methanol and water (Bruneton 1999; Scalbert *et al.,* 2005). Solvent based serial extraction system ensures separation of bioactive compounds according to their polarities. The compounds can thus be isolated, tested and progressed through the process of drug discovery and development into new drug candidates for specific diseases or derangements.

Allium cepa is one of the medicinal plants that have attracted attention worldwide (Teshika, *et al.,* 2018). This is due to the diverse of its phytochemical substances which confers various pharmacological activities such as antioxidant, anti-inflammatory, anti-diabetic, antimicrobial, analgesic and anti-alopecic effects (Khan *et al.,* 2017). There are several sub-species and cultivar of *Allium cepa* that have been studied for their medicinal benefits. Several reports on the toxicology and pharmacological activities of these *A. cepa* cultivars have been documented (Sivam 2001; Stajner & Varga 2003; Talcott 2004; Sebastian 2007; Guitart *et al.,* 2008; Montgomery 2013). Previous researchers observed that bioactivities of *A. cepa* have been on the aqueous or alcohol-based extracts (Mohammadi-Motlagh *et al.,* 2011; Nasri *et al.,* 2012; Wang *et al.,* 2013; Kalangutker and Joshi 2015). These extracts are laden with several phytochemicals without a clear identity of the bioactive principle(s) responsible for the pharmacological actions. The challenge of drug-drug interaction between these bioactive compounds in the crude extract or juice, which may either be synergistic or antagonistic cannot be overruled (Brantley *et al.,* 2014; De-Boer *et al.,* 2015).

Also, extraction affects the potency of pharmacological and toxic principles in plant extract due to increasing concentrations of these principles per unit of extract (Jones & Kinghorn 2012). Hence, the current study was carried out in investigation of bioactivity guided fractionation of red cultivar *A. cepa* L. This is to identify the bioactive principles responsible for its anti-inflammatory and analgesic effects.

MATERIALS AND METHODS

ETHICAL APPROVAL

This study was approved by the University of Ibadan, Animal Care Use and Research Ethic Committee (ACUREC) with approval number UI/ACUREC/AJO/16/0030. A specimen sample of the plant was registered and deposited at the herbarium of the Department of Pure and Applied Botany, College of Biosciences, Federal University of Agriculture, Abeokuta, Nigeria with voucher number: FUNAABH0029.

COLLECTION OF PLANT AND PREPARATION OF EXTRACTS

Fresh red cultivar *A. cepa* L bulbs, called onion in English and *alubosa* in *Yoruba* language, were purchased from a local onion market in Abeokuta, Ogun State, Nigeria. The dry coverings of the bulbs were peeled off the bulbs and the peeled onion bulbs were oven dried at 30^0C. Two hundred grams of dried *A. cepa* were blended using a Waring Laboratory Blender (ThermoScientific, USA) and stored for the serial extraction using four solvents (N-Hexane, Ethyl acetate, Chloroform and methanol).

SOLVENT PARTITIONING

The dried and granulated onion bulbs were first macerated in n-Hexane for 72 hours after which the filtrate was drained and the remains of the granules were air dried for about 24 hours in order to remove the solvent. The dried plant material was again macerated in ethyl acetate for 72 hours, the filtrate was drained and remains of the granules were air dried for about 24 hours in order to remove the solvent. The procedure was repeated for chloroform and methanol in that order using the same granulated onion bulbs. The extracts obtained from these solvents were concentrated by evaporation using a rotary evaporator (BUCHI R0210, Switzerland) and properly labelled as: N-hexane *Allium cepa* Extract; Ethyl acetate *Allium cepa* Extract; Chloroform *Allium cepa* Extract and Methanol *Allium cepa* Extract.

ANTI-INFLAMMATORY STUDY USING CARRAGEENAN-INDUCED PAW OEDEMA IN RATS

Eighty (80) rats of either sex were used in this anti-inflammatory model. The rats were randomly assigned into four groups of twenty (20) rats for each of the extracts. The rats for each of the extract were randomly and equally divided into 4 groups (A, B, C and D). Groups A and B represent the negative and positive controls, respectively. The rats in the negative control were orally administered with distilled water (10 ml/kg) while the rats in the positive control were orally treated with reference drug (indomethacin (10 mg/kg). Groups C and D were administered *A cepa* extracts at doses of 20 and 50 mg/kg, respectively. The experiment was preceded by an overnight fasting of about 12 hours. The paw size was measured using the cotton thread method (Olajide *et al.,* 2000). One hour after treatment with the extracts, distilled water or reference drug, 0.2 ml (2% w/v) carrageenan was injected into the right hind paw of each rat under the sub-plantar region. The paw sizes were measured before, immediately after injection of carrageenan (0 h) and at intervals of 0.5, 1, 1.5 and 2 hours after carrageenan injection. Cotton thread was wrapped around the paw and the length of the circumference measured with a meter rule. The inhibitory activity and percentage inhibition of inflammation by the extracts or indomethacin was then calculated as stated below.

$$\text{Percentage inhibition} = \frac{(C_1 - C_0)_{control} - (C_1 - C_0)_{treated}}{(C_1 - C_0)_{control}} \times 100$$

Where C_1 is paw size after carrageenan injection, C_0 is paw size before carrageenan injection.

ANALGESIC STUDIES USING ACETIC ACID-INDUCED ABDOMINAL WRITHING RESPONSE IN MICE

Eighty (80) mice of either sex were used in the analgesic model. The mice were randomly assigned into four groups of twenty (20) mice for each of the extracts. The mice for each of the extract were randomly divided into 4 groups (A, B, C and D). Groups A and B represent the negative and positive controls, respectively. The mice in the negative control were orally administered with distilled water (10 ml/kg) while the mice in the positive control were orally treated with reference drug (indomethacin (10 mg/kg) (Lucas 2016). Groups C and D were administered *A cepa* extracts at doses of 20 and 50 mg/kg, respectively. Following an overnight fasting, the mice were orally administered the extracts, distilled water or the reference drug. One hour after treatment with, 0.6% acetic acid (10 ml/kg) was intraperitoneally injected into each mouse. Five minutes post-injection of acetic acid, the number of abdominal constrictions were counted for the next 20 minutes and recorded. The inhibition or reduction in the number of acetic acid-induced abdominal contractions, when compared with the control groups, was indicative of analgesic effect (Siegmund *et al.*, 1957).

STATISTICAL ANALYSIS

Data of anti-inflammatory and analgesic pharmacologic models generated from this study were presented as mean ± SEM. The difference between the means in the treated groups and in the untreated groups were compared by one way analysis of variance (ANOVA) at 95% confidence interval using the Prism 5.0 Graphpad Statistic software.

RESULTS

ANTI-INFLAMMATORY STUDY

The results of anti-inflammatory study revealed that the peak percentage inhibition of paw oedema formation by the N-Hexane fraction of red cultivar *A. cepa* administered at 20mg/kg was 62.50%. This peak was attained as early as 30 min PI after which the range of inhibition was between 50.00% to 59.65% for both 20 and 50 mg/kg doses. At this point, the percentage inhibition of paw oedema by N-Hexane fraction (20 mg/kg) was significantly ($p<0.01$) higher than that of indomethacin (15%). The percentage inhibition of paw oedema by N-Hexane fraction (20 and 50 mg/kg) at 30 and 60 min PI were all significantly ($p<0.05$) than that of the indomethacin within the same period while the reverse is the case at 90 and 120 min PI (Figure 1).

The results of the inhibition paw oedema by the ethyl acetate fraction of red cultivar *A. cepa* showed that at the dose of 50 mg/kg, it reached its peak of 78.85% at 90 min PI while its 20 mg/kg reached its peak of 71.80% at 60 min PI. The percentage inhibition for both doses of red cultivar *A. cepa* at 30 min and 60 min PI were significantly ($p<0.05$) higher than that of indomethacin at the same time but the reverse is the case at 120 PI. However, there was no significant ($p>0.05$) difference between the percentage inhibition caused by administration of 50 mg/kg of red cultivar *A. cepa* at 90 min PI and that of the indomethacin within that period (Figure 2).

The chloroform fraction of the red cultivar *A. cepa* inhibited formation of paw oedema up to 74.36% with 20 mg/kg at 60 min PI. As observed in the first two extracts, the inhibition of paw oedema by chloroform fraction of the red cultivar *A. cepa* extract at 30 and 60 min PI were significantly ($p<0.01$) higher than the values of the indomethacin within the same period. Though the percentage inhibition of paw oedema by chloroform fraction of the red cultivar *A. cepa* extract at 90 and 120 min PI were high but they were significantly ($p<0.05$) lower than that of indomethacin within the same period (Figure 3).

The inhibition of paw oedema by methanol fraction of red cultivar *A. cepa* was generally low when compare with other fractions. However, it attained peak percentage inhibition of paw oedema at doses 20 and 50 mg/kg of 59.65% at 120 min PI (Figure 4).

ANALGESIC STUDY

The numbers of abdominal writhe count in mice administered with any of the fractions red cultivar *A. cepa* extract were all significantly ($p<0.05$) lower than the abdominal writhes in the control group (100.00±8.95) but significantly ($p<0.01$) higher than the abdominal writhe count in the mice administered with indomethacin (22.60±4.19) which is 78% abdominal writhe inhibition in the mice. The 50 mg/kg methanol fraction of the red cultivar *A. cepa* was the best of all the fractions with 65.67±1.20 abdominal writhes which is 35% writhing inhibition (Figure 5).

DISCUSSION

Anti-inflammatory and analgesic effects of four serially extracted fractions of red cultivar *Allium cepa* L Bulb in rats and mice were observed. The findings from the anti-inflammatory study revealed that all the extracts of red cultivar *Allium cepa* exhibited varying degrees of inhibition of paw oedema induced by the administration of carrageenan in rats. This indicates that each of these extracts possess at least a bioactive principle that have anti-inflammatory activity. The organic solvent used for the serial extraction of the red cultivar *Allium cepa* L extracts (N-Hexane, Ethyl acetate, Chloroform and Methanol) had polarity indices of 0.0, 4.3, 4.4 and 6.6 (Snyder 1974; Norlia *et al.*, 2014),

respectively. It was also observed from this study that bioactive compounds extracted with solvents on either extreme of polarity (N-hexane and Methanol) had relatively

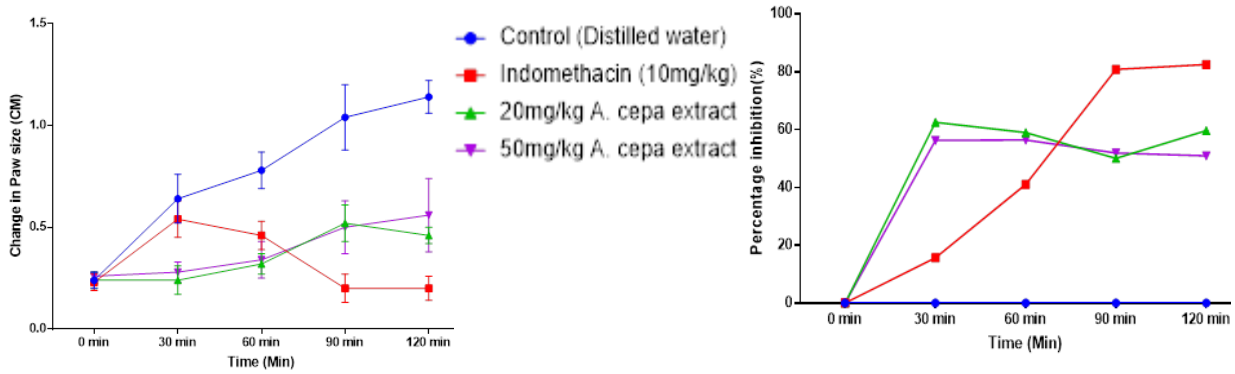

Figure I: Effect of N-Hexane solvent partitioned fraction of red cultivar *Allium cepa* L extract on change in paw sizes (cm) and percentage inhibition of oedema in rat injected with Carrageenan

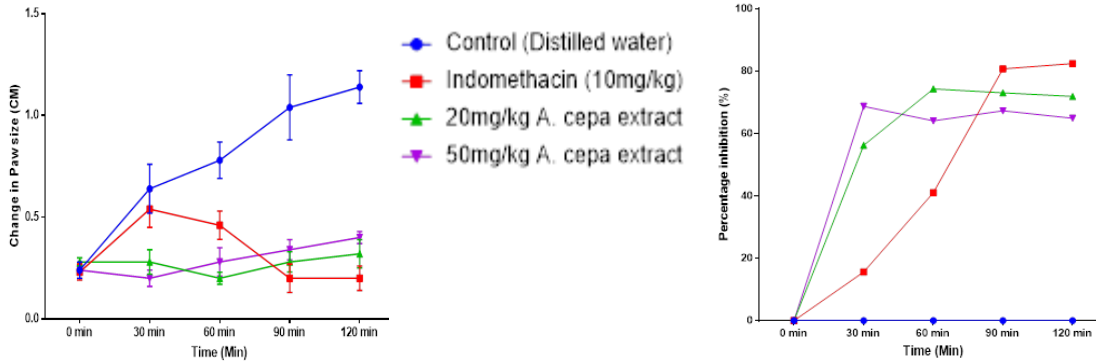

Figure II: Effect of Ethyl Acetate solvent partitioned fraction of red cultivar *Allium cepa* extract on change in paw sizes (cm) and percentage inhibition of oedema in rat injected with Carrageenan

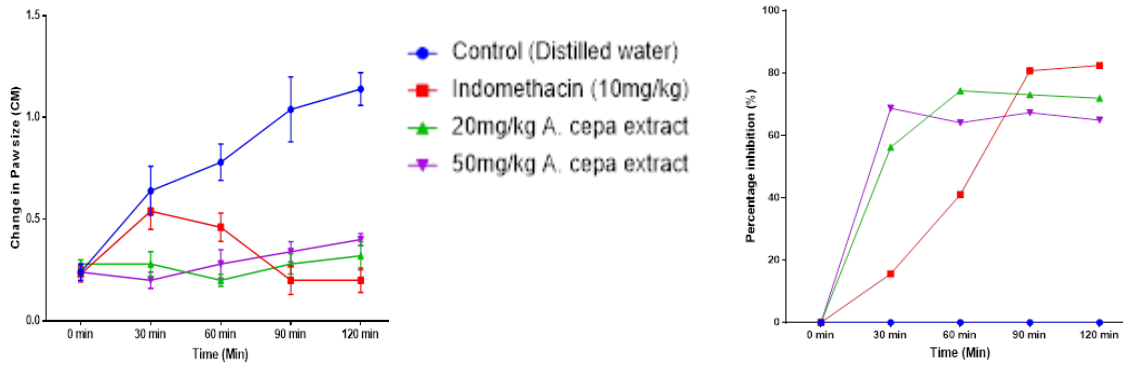

Figure III: Effect of Chloroform solvent partitioned fraction of red cultivar *Allium cepa* extract on change in paw sizes (cm) and percentage inhibition of oedema in rat injected with Carrageenan

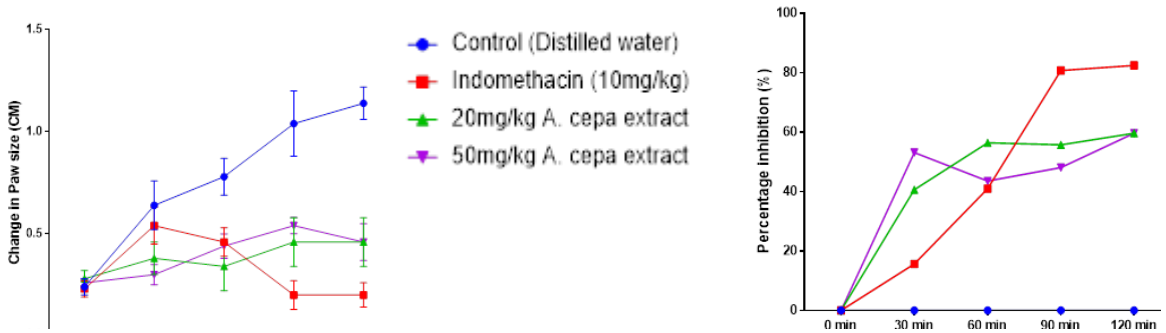

Figure IV: Effect of Methanol solvent partitioned fraction of red cultivar *Allium cepa* extract on change in paw sizes (cm) and percentage inhibition of oedema in rat injected with Carrageenan

low anti-inflammatory activities compared to the ethyl acetate and chloroform extracts. The most potent of these four extracts was the ethyl acetate extract which had higher activities and faster onset of action and maintained its potent anti-inflammatory activity till 120 min post-injection (PI). Comparatively, the anti-inflammatory capacity of chloroform extract was very close to the ethyl acetate extract as it also had high effect and faster onset of action which was relatively maintained till 120 min PI. This observation may be due to close polarity indexes of ethyl acetate (4.4) and chloroform (4.3) which may have enhanced dissolution of compounds of similar polarity indices (Green 2004) and perhaps structurally related.

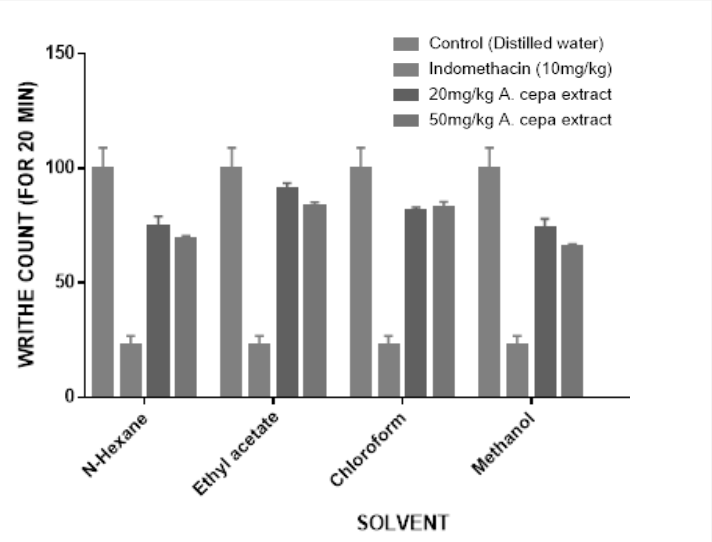

Figure V: Response of mice administered with *Allium cepa* fractions of n-Hexane, ethyl acetate, chloroform and methanol extract to acetic acid induced abdominal writhing

Oyewusi *et al.* (2021) observed highest anti-inflammatory effect of 200 mg/kg of crude methanol extract of *A. cepa* red cultivar was attained 60 minutes post injection (76.92%). The effect was not maintained because it declined to 68.42% at 120 PI. The present result showed that even 20 mg/kg and 50 mg/kg of ethyl acetate and chloroform fractions attained very high anti-inflammatory effect at 30 minutes PI which was maintained till 120 minutes PI. This implies that solvent fractionation of the extracts has positive influence on the anti-inflammatory activities of the extracts.

The bioactive metabolites in ethyl acetate and chloroform extracts of red cultivar *Allium cepa* L. extracts may have contained compounds of medium polarity indices, possessing functional groups that are capable of inhibiting certain inflammatory processes as obtainable with many other anti-inflammatory substances of plant origin (Furst & Zundorf 2014). Their mechanism of action may occur in similar way as it is with Non-steroidal anti-inflammatory drugs (NSAIDs). In order to exhibit effective anti-inflammatory effects, the metabolites should be able to inhibit or modulate or control one or two or any of the following processes of inflammatory reactions. This include inhibition of certain pro-inflammatory signal cascades such as NF_KB, COX I and II (Hong *et al.,* 2004; Kim *et al.,* 2005; Pushpangadan *et al.,* 2015) and down-modulation of secretion of certain cytokines such as TNFα, IL-1β and IL-6 (Shah *et al.,* 2010). Also, modulation of cellular activities of inflammatory cells like mast cells, lymphocytes, neutrophils and macrophages (Pushpangadan *et al.,* 2015) as well as regulation of pro-inflammatory gene expression or blockage of cell adhesion molecules necessary for leukocytes with endothelial cells (Kumar *et al.,* 1998; Bellik *et al.,* 2013) are important inflammatory reactions.

The results of the analgesic study with the extracts showed that none of the extracts exhibited appreciable analgesic activity when compared with the indomethacin. This indicates that none of the fractions of the red cultivar *Allium cepa* L. extracts at this stage of extraction had strong analgesic activities. The analgesic result in this present study corroborates with the previous research of Oyewusi *et al.* (2021) in which red cultivar of *A. cepa* crude methanol extract was found to exhibit mild analgesic activities. This result is also in agreement with Owoyele *et al.* (2006) who reported that *A. cepa* extracts have mild analgesic but strong anti-inflammatory effects. This implies that solvent fractionation of the extracts has no influence on the analgesic activities of the extracts.

The method of serial extraction used in this study, in which solvent of different polarity were used in succession starting from solvent of lowest polarity index to the solvent of highest polarity ensured a wide range of bioactive metabolites separated according to their polarity index. In so doing, the possibility of drug-drug or bioactive interaction is reduced. Subjecting each of these extracts to tests revealed the polar region where the most active compound with the evaluated bioactivities resides. The compound(s) responsible for the bioactivity can then be isolated from the fraction that exhibited the highest effect. For this research, the fraction extracted with ethyl acetate was the most effective for anti-inflammatory test while methanol extract was the best for analgesic test. Previous reports on several species of *Allium* revealed potent anti-inflammatory and analgesic effects due to the presence of flavonoids and organosulphur compounds (Ranjan *et al.,* 2010). Due to paucity of imformation/ data on the anti-inflammatory and analgesic activities red cultivar *Allium cepa* L. solvent fractions. However, in a study carried out using related plant, *Allium stracheyi* by Ranjan *et al.,* (2010),

the methanol fraction exhibited the highest anti-inflammatory and analgesic actions with 61.00% and 64.62%, respectively. In the case of Aslam *et al.* (2017), the extractions extracts of *Allium fistulosum* were done using ethanol, methanol and water in that other and ethanol extract was found to exhibit highest anti-inflammatory effect compared to others. This also does not compare well with the current results because of difference in plant species and the solvents polarity.

CONCLUSION

In conclusion, the results in this study show that every extracted metabolite regardless their polarity position had both anti-inflammatory and analgesic effects exhibited at different levels of effects. The study also showed that red cultivar *Allium cepa* L. had very high anti-inflammatory activity but weak analgesic activity compared to indomethacin. The medium polar compounds in the ethyl acetate fraction which showed the most potent anti-inflammatory activity is suggested for further fractionation, isolation of bioactive metabolites and chemical elucidation of probable anti-inflammatory drug candidate(s). These drug candidates can further be tested for treatment of diseases of inflammatory origin such as hypertension, diabetes and cancers.

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