In Vitro Anti-inflammatory and Antimicrobial Activities of *Acalypha godseffiana* Leaf Extracts

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Abstract

Acalypha godseffiana has been traditionally used for the treatment of various ailments, and previous study reported phytochemicals with promising antioxidant properties. This study was carried out to investigate the in vitro anti-inflammatory and antimicrobial activities of the leaf extracts of A. godseffiana. The antimicrobial activities were determined by agar well diffusion method while anti-inflammatory potential was assessed by assessing inhibition of albumin denaturation, membrane stabilization, and antiproteinase activity. It was observed that the methanol and aqueous extracts exhibited both anti-inflammatory and antimicrobial activities that varied between the microbial species with the methanol extract showing maximum antimicrobial activity against Staphylococcus aureus (18 mm zone of inhibition) but susceptibility test with Aspergillus niger produced the least inhibition denoting the lowest antimicrobial activity. This study shows that methanol extracts exhibited a higher antimicrobial and in vitro anti-inflammatory activity when compared with the aqueous extracts. The anti-microbial activity of the A. godseffiana extract was found to be comparable to the antimicrobial positive controls for some bacterial (E. coli and K. pneumoniae) and the fungal isolates used as test organisms. The study revealed that A. godseffiana possesses anti-inflammatory and antimicrobial properties signifying it could possess active phytochemicals and functional bioactive compounds that could warrant these purposes. Therefore, this plant could be exploited for the treatment of inflammation and biological systems susceptible to free radical-mediated reactions due to the significant percentage of inhibition of protein denaturation as well as its proof of good antimicrobial activities. However, further studies are required to characterize the active constituents responsible for the anti-inflammatory and antimicrobial activities and to confirm their mechanism of action.

Keywords: *Acalypha godseffiana*, Anti-inflammatory, Antimicrobial, *In Vitro*, leaf extracts.

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Introduction

Inflammation may not be listed as a disease by the World Health Organization (WHO) but it is a major cause of death to the world population (WHO, 2001). Inflammation is a consequence of diseases, such as cardiovascular diseases, diabetes, rheumatoid arthritis, neurodegenerative disorders, premature ageing, cancer, and others, when humans are exposed to biological, environmental and/or chemical agents such as pathogens, damaged cells, and others (Jagetia *et al*., 2004). This is because inflammation has been implicated in elevated levels of nitric oxide (NO) formation, autoxidation of lipids and oxidative stress which may bring deleterious effects, metabolic dysfunction, degenerative or pathological processes and numerous disease states (Huang *et al.,* 2005). Inflammation is characterized by pain, heat, redness, swelling and loss of function (Anoopa-John *et al*., 2020). Since most underdeveloped and developing communities lack access to orthodox medicines, they depend on the use of decoctions or herbal mixtures (traditional medicine) for their cure (Odediran *et al.,* 2014; Olubodun and Osagie, 2018). These mixtures, being combinations, are believed to combat ailments in multiple dimensions to relieve sufferers undergoing treatment, just as orthodox medicine, employ combination therapy is the treatment of some ailments (Odediran *et al.,* 2014; Olubodun and Osagie, 2018).

Inflammation is a mechanism of innate immunity (Anoopa-John *et al*., 2020). Oxidative stress, autoxidation process and elevated levels of NO may result in the formation of potentially toxic compounds, leading to health issues such as aging, cardiovascular disease, diabetes, and cancer (Truong *et al.,* 2019). Oxidative stress usually occurs when the antioxidants defense is overwhelmed by either endogenous or exogenous oxidants or free radicals called reactive oxygen species (ROS) or active oxygen species (AOS). There are different types of activated oxygen, which include free radicals such as superoxide ions (O^{2-}) and hydroxyl radicals (OH), as well as non-free radical species such as hydrogen

peroxide (H_2O_2) (Yildrim *et al*., 2001; Olubodun and Osagie, 2018). Reaction oxygen species may be a result of normal endogenous sources such as aerobic respiration, macrophages, perioxisomes or exogenous sources of free radicals such as ionizing radiation, certain pollutants, including pesticides, crude oil (Yildrim *et al*., 2001; Olubodun and Osagie, 2018).

Free radical formation is scavenged and minimized by antioxidant defense system (Jain *et al.*, 2006; Olubodun and Osagie, 2018). Synthetic antioxidants have been extensively used to avoid the oxidative stress and autoxidation process. However, the use of synthetic antioxidants is restricted due to safety concerns (Akinmoladun *et al.*, 2007; Truong *et al.*, 2019). Then the search for natural agents as alternatives to synthetic antioxidants became a challenge to scientists (Złotek *et al.*, 2016; Truong *et al.*, 2019), who are researching tirelessly, on the use of antioxidants (flavonoids, phenols, tannins, and alkaloids) from plants as ameliorating and therapeutic agents.

The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. These antioxidant phytochemicals have the ability to quench free radicals, chelate catalytic metals and scavenge oxygen (Złotek *et al.*, 2016; Truong *et al.*, 2019). The antioxidant potentials of the plants have been shown to prevent oxidative stress, autoxidation, disease processes and maintain health (Olubodun *et al*., 2021a). There are reports that these antioxidants also possess anti-inflammatory potentials which protect the body against inflammation, thus may prevent diseases and disorders caused by inflammation (Moreno-Quiros *et al.*, 2017; Truong *et al.*, 2019).

Plants have been identified all over the world as good sources of medicinal agents. Africa is blessed with varieties of natural vegetation used in traditional medicine to treat and cure different tropical ailments ranging from inflammation, fungal and bacterial infections, and others (Noumedem *et al.*, 2013; Olubodun *et al.*, 2021a). One of the plants used for medicinal purposes in

Africa, particularly in Nigeria is *Acalypha godseffiana*. It belongs to the *Euphobiaceae* family in plant nomenclature or scientific classification. It is a shrub growing in the rainforest region and is used as an ornamental crop (Falodun *et al.*, 2007). The leaves, commonly called copper leaf or Joseph's coat because of its mixed colours, and broad with a width of about 11-13 cm (Falodun *et al.*, 2007; Ezekiel *et al.*, 2009).

A. godseffiana is used as a traditional medicine to treat skin infections, malaria, stomach pain and other ailments (Falodun *et al.*, 2007; Ezekiel *et al.*, 2009). Previous studies (e.g., Evanjelene and Natarajan, 2012) have reported the presence of phenol, tannins, alkaloids, flavonoids and saponins in the leaf extracts of *A. alnifolia*. The ethanolic extract of *A. wilkesiana* leaves revealed the presence of tannins, steroids, flavonoids and cardiac glycosides while saponins, alkaloids and anthraquinones were not present (Gotep *et al*., 2010). The antibacterial, antifungal and/or antioxidant activities of *A. monostachya, A. wilkesiana, A. indica, A.alnifolia, A. hispida* were also reported in several studies by Evanjelene and Natarajan (2012), Noumedem *et al.* (2013), and Olubodun *et al.* (2021a). The health benefits of *Acalypha sp* lies in their phytochemicals such as flavonoids, phenols, tannins and saponins (Noumedem *et al.*, 2013; Olubodun *et al.*, 2021a). These phytochemicals have been found to possess various biological activities such as antiallergic, antibacterial, antimalarial, antivirus, antioxidant, and anti-inflammatory activities (Noumedem *et al.*, 2013; Truong *et al.*, 2019; Olubodun *et al.*, 2021a). Since *A. godseffiana* have been widely reported to be a good source of antioxidants and with antimicrobial properties, (Olubodun *et al.*, 2021a), it may therefore be a promising anti-inflammatory and antimicrobial agents and these justify the aim of this study to investigate the antimicrobial and anti-inflammatory activity of the leaf extracts of *A. godseffiana*.

Materials and Methods

Chemicals. All chemicals and reagents such as Methanol, phosphate buffer, nutrient broth, Dimethyl sulfoxide (DMSO), *Chloramphenicol*, *Fluconazole*, bovine serum albumin (BSA), casein, perchloric acid, aspirin, Tris-HCl, and trypsin were analytical grade and purchased from either Sigma-Aldrich, UK and/or May and Baker, UK.

Collection, Identification and authentication of Plant Material. Fresh leaves of *Acalypha godseffiana* were obtained from the Ugbowo campus of the University of Benin, Benin City, Nigeria. The plant was initially identified based on its phenotypic or observable features and further authenticated by a certified botanist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

Collection, Purification and Standardization of test organisms

The bacterial and fungal pathogens used were obtained from the Department of Microbiology, University of Benin, Benin City, Nigeria. The bacterial isolates were first sub-cultured in a nutrient broth (Sigma) and incubated at 37 °C for 18 hours. The fungal isolates were sub-cultured on potato dextrose agar (PDA) (Sigma) for 7 days at 25 °C.

Positive and negative controls Chloramphenicol (100 µg/mL) and Fluconazole (100 µg/mL) were used as positive controls for the antibacterial and antifungal tests, respectively. DMSO was used as negative control for the antibacterial and antifungal analyses.

Preparation of A. godseffiana Leaf Extracts. The leaves of *A. godseffiana* was dried at room temperature for a period. It was reduced to fine powder after showing brittle properties when crushed with hand using a mechanical blender. The powdered sample (1000 g) was soxhlet extracted with 2000 mL of methanol,

then concentrated with a rotary evaporator at reduced pressure (Naz *et al*., 2017). The concentrated extract was stored in a refrigerator at 4 ^{o}C until required for further studies.

Another quantity of the fresh leaves of *A. godseffiana* was macerated with distilled water (250 mL) for 48hours. The extract was filtered and concentrated to dryness with a rotary evaporator in vacuo. The dried powdery sample was stored at 4^{o}C until required for analysis.

Determination of Antimicrobial Activity. The antimicrobial activity of the leaf extract of *A. godseffiana* was determined by the agar well diffusion method and minimum inhibitory concentration (MIC).

i). *Assay for antibacterial and antifungal activity.* The antibacterial and antifungal activity of the leaf extracts of *A. godseffiana* were determined by the agar well diffusion method as described by Aladejimokun *et al.* (2017), with slight modifications, using 0.1 mL of antibiotic chloramphenicol (100 µg/mL) for bacteria and Fluconazole (100 µg/mL) for fungi test organisms as positive controls in separate wells.

One millilitre of standardized inoculum (1.5× 108 CFU/mL) of each bacterium was separately added to 20 ml of sterile molten nutrient agar in sterile Petri-dishes. The contents were mixed and allowed to solidify. Wells of 5mm diameter were bored using a sterilized cork borer; thereafter 0.1 ml of the extracts (methanol, 100 µg and aqueous, 100 µg), prepared by diluting 1.0 g of the extracts in 100 mL of 0.01% Tween-20 to obtain a concentrations of 100 µg/mL and 0.1 mL of antibiotic, chloramphenicol (100 µg/mL) for bacteria and myconazole (100 µg/mL) for fungi test organisms as positive controls in separate wells. The plates were allowed to stand at room temperature for 10 minutes to allow the diffusion of the extracts into the agar. The plates were then incubated at 37°C for 24 hours for bacteria and 72 hours for fungi.

After incubation, the diameters of the zones of inhibition around the wells were measured in millimetres (mm) (Opawale *et al.*, 2015).

ii). *Determination of minimum inhibitory concentration (MIC).* The MIC of the leaf extracts were determined by the microdilution method as described by Naz *et al*. (2017), using nutrient broth. The leaf extracts were dissolved in 10% DMSO and two-fold dilutions were prepared with culture broth. Each test sample and growth control (containing broth and DMSO, without leaf extracts/antimicrobial substance) was inoculated with 10 µL of bacterial suspension containing 5 × 106 CFU/mL. A 10-µL solution of resazurin (270 mg resazurin tablet dissolved in 40 mL of sterile water) was also added to each sample and incubated for 24 hours at 37 °C. Bacterial growth was detected by reading absorbance at 500 nm. Bacterial growth was indicated by a color change from purple to pink or colorless (assessed visually). MIC was defined as the lowest plant extract concentration at which the color changed, or the highest dilution that completely inhibited bacterial growth.

***Determination of In Vitro Anti-Inflammatory Activity.* :** The *in vitro* anti-inflammatory activity of *A. godseffiana* leaf extracts was carried out by assessing inhibition of albumin denaturation, membrane stabilization, and antiproteinase activity as described by Truong *et al.* (2019), with slight modifications to method applied. Both extracts and the standards were tested at 10 µl/mL concentration. Aspirin (100 μg/mL), was used as positive control, while Dimethyl sulfoxide (DMSO) was used as negative control.

i). *Inhibition of Albumin Denaturation.* To prepare the reaction mixture, 1 mL of 1% aqueous solution of bovine albumin fraction was added into 1 mL of tested extract. The pH of reaction mixture was then adjusted to 6.3 before being incubated for 20 minutes at 37°C and heated to 51°C for 30 minutes. After cooling to room temperature, the absorbance of the sample was measured at 660 nm.

The percentage inhibition of protein denaturation was calculated using the equation:

Percentage inhibition = $(A_C - A_S)/Ac \times 100$

where A_C is absorbance (660 nm) of control and A_S is absorbance (660 nm) of sample. The results were reported as IC_{50} values (concentration required for a 50% inhibition).

ii). ***Antiproteinase Activity.*** One mL sample was added into reaction mixture containing 1 mL of 20 mM Tris HCl buffer (pH 7.4) and 0.06 mg trypsin. The mixture was then incubated for 5 minutes at 37°C before adding 1 mL of 0.7% (w/ v) casein. The reaction mixture was incubated for an additional 20 minutes and 2 mL of 70% perchloric acid ($HClO_4$) was added to stop the reaction. The reaction mixture was centrifuged at 6,000 rpm and at 4°C for 10 minutes to collect the supernatant. The absorbance of the supernatant was then checked at 210 nm using a spectrophotometer. The percentage inhibition of proteinase was calculated using equation given in inhibition of albumin denaturation, and the results were reported as IC_{50} values.

iii). ***Membrane Stabilization.*** A blood sample was obtained from a healthy human volunteer who did not use any nonsteroidal anti-inflammatory drugs for 2 weeks. The blood cells were centrifuged at 3000 rpm for 10 minutes, washed with normal saline (3 times), and reconstituted as 10% suspension in normal saline. For the heat-induced haemolysis assay, 1 mL of sample was mixed with 1 mL of 10% human red blood cell (HRBC) suspension and incubating for 30 minutes at 56°C. The mixture was then cooled and centrifuged at 2500 rpm for 5 minutes to obtain the supernatant. The absorbance of the supernatant was then checked at 560 nm using a spectrophotometer. The percentage inhibition of haemolysis was calculated using equation given in inhibition of albumin denaturation, and the results were reported as IC_{50} values.

Statistical Analysis. All analyses were done in triplicate, and average values recorded as mean plus and minus their standard error of mean (±SEM). Instat-Graphpad software, Prism 6, San Diego, California, USA, was used to carry out all analyses. Statistical comparisons were carried out by analysis of variance (ANOVA) Tukey's multiple comparison test, and p values < 0.05 were considered as statistically significant.

Results

Antimicrobial Activity of *A. godseffiana* leaf extracts.

The antimicrobial activities of the leaf extract of *A. godseffiana* against the test pathogens is presented in Tables 1 and 2. The results shows that the leaf extract exhibited antimicrobial activity against all the microorganisms tested. The result in Table 1 showed that antibiotic control showed mean zones of inhibition ranging from 14 to 26 mm, while the fungal control recorded 10 and 12 mean zones of inhibition in the agar well diffusion assay. Even though the antimicrobial activity of the leaf extract showed lower zones of inhibition when compared with the antibiotic control or fungal control (Table 1), purification of the active secondary metabolites may produce a more active ingredient than the crude extract.

The results show that the leaf extract of *A. godseffiana* have active phytochemicals which are able to inhibit pathogenic bacteria and fungi. The results show that *B. subtilis* and *S. aureus* (Gram (+) bacteria) were more susceptible than *E. coli* and *K. pneumoniae* (Gram (-) bacteria) to the methanol extract than to the aqueous extract. The MIC values ranged between 0.06 and 5.23 mg/ml for bacteria, and from 0.10 to 1.25 mg/ml for fungi. While *S. aureus* and *A. niger* (MIC= 0. 68 - 0. 85 mg/ml) were the most sensitive microorganisms, *B. subtilis* (MIC= 3.62 – 5.23 mg/ml) was the most resistant. The lowest MIC value recorded (MIC = 0.06 - mg/ml) was from the aqueous fraction on *E. coli* and *K. pneumoniae*.

Anti-Inflammatory Activity of *A. godseffiana* leaf extracts.
The leaf extract inhibited albumin denaturation, hemolysis of HRBCs and proteinase activity as shown in Table 3. Data description of each parameter for anti-inflammatory activity are related under sub-headings below:

***Inhibition of Albumin Denaturation*:** The results in Table 3 shows the inhibitory effect of the methanol and aqueous extracts of *A. godseffiana* on albumin (protein) denaturation. However, methanol extract recorded a higher inhibitory activity (75%) against albumin denaturation when compared with the aqueous extract which produced 51% inhibitory activity. The methanol extract exhibited a relatively higher but non-significant protein protection as compared with aspirin. This is because the result observed that aspirin inhibited approximately 73% of albumin denaturation at a concentration of 0.2mg/mL when compared to the methanol extract but a significantly lower protein protection in the aqueous extract.

***Antiproteinase Activity*:** The anti-inflammatory activity of *A. godseffiana* leaf extracts measured by antiproteinase activity is also presented in Table 3. A higher antiproteinase activity was also recorded in the methanol extract when compared with the aqueous extract (Table 3). Both extracts (methanol and aqueous) however, demonstrated a significantly lower inhibitory activity against proteinase than aspirin, though inhibitions produced were above average percentage.

Membrane Stabilization: Table 3 also showed the anti-inflammatory activity of *A. godseffiana* leaf extract based on membrane stabilization by the ability to protect HRBCs from heat-induced haemolysis. The methanol extract showed a higher inhibition against heat-induced haemolysis than the aqueous extract as shown in Table 3. The results shows that the extracts may protect the membrane against haemolysis induced by heat. When compared with aspirin (82%), the results of the study revealed that the

membrane stabilizing effect of *A. godseffiana* extracts is an additional mechanism for their anti-inflammatory activity.

Table 1: Antimicrobial activity of methanol and aqueous extracts of *Acalypha godseffiana on microbial isolates* using agar-well diffusion method.

Organisms	Zones of inhibition (mm) Methanol (100 µg)	Aqueous (100 µg)	Chl (100 µg)	FL (100 µg)
SA	18	17	26	-
BS	17	16	22	-
EC	14	12	14	-
KP	15	14	16	-
AN	11	10	-	12
CA	15	12	-	10

SA = *Staphylococcus aureus*; BS = *Bacillus subtilis*;
EC = *Escherichia coli*; KP = *Klebsiella pneumoniae*;
AN = *Aspergillus niger*; CA = *Candida albicans*;
Chl = *Chloramphenicol*; FL = *Fluconazole*; - = No inhibition

Table 2: Minimum Inhibitory Concentration of extracts of *Acalypha godseffiana*

Organisms	Methanol (mg/mL)	Aqueous (mg/mL)
SA	0.68 ± 0.11	1.33 ± 0.12
BS	5.23 ± 0.19	3.62 ± 0.11
EC	2.87 ± 0.07	0.06 ± 0.10
KP	2.50 ± 0.04	0.09 ± 0.15
AN	0.85 ± 0.33	0.10 ± 0.10
CA	1.25 ± 0.19	0.10 ± 0.12

S.A = *Staphylococcus aureus*; BS = *Bacillus subtilis*;
EC = *Escherichia coli*; KP = *Klebsiella pneumoniae*;
AN = *Aspergillus niger*; CA = *Candida albicans*

Table 3. Effect of *A. godseffiana* leaf extracts on albumin denaturation, membrane stabilization and proteinase inhibition.

Extract /control	Albumin denaturation % inhibition	HRBCs Membrane stabilization % inhibition	Proteinase inhibition % inhibition
Methanol extract	75.48 ± 0.05^{a}	65.09 ± 0.03^{b}	69.76 ± 0.06^{b}
Aqueous extract	51.09 ± 0.03^{b}	54.42 ± 0.05^{c}	61.64 ± 0.06^{b}
Aspirin (0.2 mg/mL)	72.58 ± 0.12^{a}	81.89 ± 0.09^{a}	90.67 ± 0.05^{a}

All experiments are in three replicates with values of Mean ± standard error of mean (SEM). The values carrying different superscripts in the same column are significantly different at $P<0.05$.

Discussion

The use of plant as a natural source of secondary metabolites to promote health and wellness has been attracting attention in the past decades. The study used distilled water (aqueous) and methanol to extract the secondary metabolites from *A. godseffiana* leaves. An earlier report by Olubodun *et al*. (2021a), recorded a higher extraction yield for methanol extract when compared with the aqueous extract. Olubodun *et al*. (2021a), also reported that *A. godseffiana* leaves contains alkaloids, tannins, flavonoids, terpenoids, steroids and phenolic compounds which may have contributed to their antioxidant activity.

The results show that the leaf extract exhibited antimicrobial activity against all the microorganisms tested. The results of the antimicrobial activity show that the leaf extract of *A. godseffiana* have active phytochemicals which are able to inhibit pathogenic bacteria and fungi.

The results show that *B. subtilis* and *S. aureus* (Gram (+) bacteria) were more susceptible than *E. coli* and *K. pneumoniae* (Gram (-) bacteria) to the methanol extract than to the aqueous extract. Some

researches recorded similar reports for other plant species (Noumedem *et al.*, 2013; Eshwarappa *et al.*, 2016; Moharram *et al*., 2017; Naz *et al*., 2017; Truong *et al.,* 2019), making the antimicrobial activities of *A. godseffiana* comparable to other plants and may be added as another plant in the bank of herbal medicines. However, the result is at variance with the report of Govindappa *et al.* (2011), who reported that the ethanol stem extract of *Wedelia trilobata* inhibited the growth of almost all the bacteria isolates but did not show any significant effect on fungal isolates.

The sensitivity of the microorganisms to the leaf extract may be attributed to the differences in their cell wall composition and/or in genetic content of plasmids that may be transferred among microbes (Noumedem *et al.,* 2013; Eshwarappa *et al.,* 2016; Aladejimokun *et al.*, 2017; Moharram *et al.,* 2017; Naz *et al*., 2017; Truong *et al,*. 2019). Also, we may attribute the susceptibility to higher extraction yield recorded for the methanol extract which may have extracted more antimicrobial and anti-inflammatory constituents when compared with the aqueous extract. In the case of the fungi, *A. niger* was more susceptible to the extracts than *C. albicans*. This may have resulted from the differences in the morphology, physiological and biochemical make up. It may also have been a case of the differences in the mechanism by which the secondary metabolites of the leaf extract exerted their effect. This variation was also observed in other plant species by other researchers (Noumedem *et al.,* 2013; Aladejimokun *et al.,* 2017; Moharram *et al.,* 2017; Truong *et al.,* 2019). The flavonoids, phenolics, saponins, tannins and terpenoids from plant extracts have been shown to possess potent antimicrobial, antioxidant and anti-inflammatory activity in various studies (Govindappa *et al.,* 2011; Olubodun *et al.,* 2021a, b).

The reaction of body tissues to infection and/or other injuries is a complex process associated with inflammation that may result in diabetes, arthritis, neurodegenerative disorders, cancer, etc (Oyeleke *et al.,* 2018; Truong *et al.,* 2019). Treatment of

inflammatory diseases using steroidal and nonsteroidal anti-inflammatory drugs are being discouraged because of their adverse health effects (Truong *et al.,* 2019). So, efforts are being made to use secondary metabolites in plants because they are relatively safe (Truong *et al.,* 2019).

Denaturation of proteins is regarded as a major cause of inflammation and degenerative diseases (Moharram *et al.,* 2017; Anoopa-John *et al*., 2020). Several anti-inflammatory drugs like salicylic acid have shown dose dependent ability to inhibit thermally induced protein denaturation (Moharram *et al.,* 2017). The *in vitro* anti-inflammatory activity of *A. godseffiana* leaf extract evaluated in this study, showed that methanol and aqueous extracts, significantly inhibited albumin denaturation. They also have protein protective capabilities, indicating that *A. godseffiana* leaf extracts have potential as novel anti-inflammatory agents.

Results of the anti-proteinase activity revealed that the leaf extracts exhibited a potential inhibitory activity against proteinase. Also, methanol extract of *A. godseffiana* effectively protected the HRBC membrane (i.e., HRBC membrane has a similar structure to the lysosomal membrane) against heat-induced haemolysis, indicating that the extract may stabilize lysosomal membranes. It is possible that the secondary metabolites in the extracts protect lysosomal membranes against injury by interfering with activation of phospholipases. The results (Table 3) thus provide evidence for the anti-inflammatory activity of *A. godseffiana* extracts through a membrane stabilization effect. This effect may possibly inhibit the release of lysosomal content of neutrophil at the site of inflammation. The extracts inhibited the heat induced hemolysis of HRBCs to varying degree (Table 3). This finding agrees with those reported by Govindappa *et al.* (2011) which stated that the fresh leaf extract followed by the fresh flower and fresh stem was effective in inhibiting heat induced albumin denaturation and antiproteinase activity. Also, the biological activity may be attributed to the presence of phytochemicals like tannins, phenols, flavonoids, saponins, and terpenoids in the leaves (Yadav *et al.,*

2014; Moharram *et al.,* 2017). The main action of the anti-inflammatory agent may be the inhibition of the cyclooxygenase system which is responsible for the biosynthesis of prostaglandins. Nonsteroidal anti-inflammatory drugs (NSAIDS) like prostaglandins acts by inhibiting the lysosomal enzymes or by stabilizing the lysosomal membrane (Govindappa *et al.,* 2011; Moharram *et al.,* 2017; Naz *et al.,* 2017; Truong *et al.,* 2019).
The lysosome may undergo lysis during the inflammatory process and release compounds that may generate inflammation that may produce several disorders. Stabilization of membrane is a process of maintaining the integrity of the lysosomal membrane against heat-induced haemolysis, which prevents the release of fluids and serum proteins into the tissues caused by inflammatory mediators, thus inhibiting the inflammatory response (Moharram *et al.,* 2017; Oyeleke *et al*., 2018; Truong *et al.,* 2019).

Conclusion

The study provided evidence that *A. godseffiana* possesses anti-inflammatory and antimicrobial properties which may be attributed to its phytochemicals/secondary metabolites able to inhibit the growth of some Gram-positive and Gram-negative bacteria and some fungi isolates. Besides, it may be used in the treatment of inflammation and biological systems susceptible to free radical-mediated reactions due to the significant percentage of inhibition of protein denaturation. The study also suggest that the leaves should be used as alternative or supplementary remedy for the prevention and/or treatment of pain and inflammatory diseases. However, further studies are required to characterize the active constituents responsible for the anti-inflammatory and antimicrobial activities and their mechanism of action.

Conflicts of Interest

The authors declare no conflicts of interest.

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