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In vitro antiviral, antioxidant and *in vivo* antipyretic activity of three South Africa medicinal plants crude extracts

[Actividad antiviral, antioxidante y antipirética *in vivo* de tres extractos crudos de plantas medicinales de Sudáfrica]

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Abstract: This study investigated anti-viral, antioxidant activity and anti-pyretic of crude extract from *Artemisia afra*, *Artemisia absinthium* and *Pittiosporum viridflorum* leaves. The crude extracts were prepared by maceration using aqueous, methanol and dichloromethane respectively. Antiviral studies were evaluated with influenza virus using Fluorescence based - Neuraminidase inhibitors. Antioxidant activities determined with DPPH, Nitric oxide, hydroxyl and super oxide anion radicals' Anti-pyretic activities were evaluated using rats with yeast induced pyrexia. Total phenol, flavonoids, and pro-anthocyanin contents of the plants samples were evaluated using standard protocols. The crude extracts exhibited neuraminidase inhibitory activity against the influenza virus at different thresholds. *Artemisia absinthium* aqueous extract showed the best activity against A/Sydney/5/97. Whereas *Artemisia afra* methanol crude extract displayed highest antioxidant potential against the tested antioxidant parameters. All the crude extracts significantly reversed yeast induced pyrexia in rats, similar to paracetamol. Thus, they could serve as natural remedy for respiratory diseases such as Influenza.

Keywords: Antiviral; Antioxidant; *Artemisia afra*; *Artemisia absinthium*; *Pittosporum viridiflorum*.

Resumen: Este estudio investigó la actividad antiviral, antioxidante y antipirética del extracto crudo de hojas de *Artemisia afra*, *Artemisia absinthium* y *Pittiosporum viridflorum*. Los extractos crudos se prepararon mediante maceración utilizando metanol acuoso y diclorometano respectivamente. Los estudios antivirales se evaluaron con el virus de la influenza utilizando inhibidores de neuraminidasa basados en fluorescencia. Actividades antioxidantes determinadas con DPPH, radicales aniónicos de óxido nítrico, hidroxilo y superóxido. Las actividades antipiréticas se evaluaron utilizando ratas con pirexia inducida por levaduras. El contenido total de fenol, flavonoides y proantocianina de las muestras de plantas se evaluó utilizando protocolos estándar. Los extractos crudos mostraron actividad inhibidora de neuraminidasa contra el virus de la influenza en diferentes umbrales. El extracto acuoso de *Artemisia absinthium* mostró la mejor actividad contra A/Sydney/5/97. Mientras que el extracto crudo de *Artemisia afra* metanol mostró el mayor potencial antioxidante contra los parámetros antioxidantes probados. Todos los extractos crudos revirtieron significativamente la pirexia inducida por levaduras en ratas, similar al paracetamol. Por tanto, podrían servir como remedio natural para enfermedades respiratorias como la Influenza.

Palabras clave: Antivírico; Antioxidante; *Artemisia afra*; *Artemisia absinthium*; *Pittosporum viridiflorum*.

INTRODUCTION

Viral infections account for substantial morbidity and mortality worldwide. World health organisation (WHO) estimates that over 500,000 people die globally of viral infection cases yearly (Wei *et al*., 2015). Emergence of viral respiratory infections including influenza, serious airway respiratory syndrome (SARS), middle east respiratory syndrome (MERS) and most recently, human coronavirus (Covid-19) among others have heightened the awareness of viral pandemics (Liu *et al.,* 2017a). Continuous emergence of new virus strains indicate we could face unpredicted situations in preventing infections in the nearest future. This spurs the need to strategize plans to mitigate virus's insurgence (Kalyani & Kamaruz, 2013).

Virus hijack host metabolic activities to promote its replications (Liu *et al.,* 2017a). Likewise, the host environmental factors like antioxidant defence system, body temperature among others directly or indirectly influences the virus's virulence (Liu *et al.,* 2017a). Oxidative stress aggravates neutrophilic infiltration, protease secretion, accumulation of oxidative intermediates and immune response, known as "cytokine storm" (Wei *et al.,* 2015). Hyperpyrexia (fever) caused by viral infections hamper normal body system functionalities, thus promote severity of the infections (Tomiyama *et al.,* 2015). Therefore, multi-therapeutic drugs become desirable to attenuate virus virulence's drivers.

Antiviral chemotherapy drugs, neuraminidase inhibitors (Zanamivir and oseltamivir) and adamantanes (rimantadine and amantadine) are commonly used to combat viral infections and their complications. Unfortunately, they are expensive and also associated with undesirable side effects including dizziness, coughing, constipation, headache, nausea, asphyxia among others (Wei *et al.,* 2015). In addition to this, vaccine against viral infection had proven to be less effective due to frequent virus antigen drift (Liu *et al.,* 2017). Hence, the search for novel antiviral agents with multi-therapeutic potentials is imperative. Natural products, especially those that are used in traditional medicine, are regarded as effective, cheaper and safer remedy against various diseases (Osunsanmi *et al.,* 2019). Coupled with their broader spectrum of biological activities, medicinal plants remain the frontline therapy to over-ride drugs resistant virus strains (Ogbole *et al.,* 2018).

South Africa is endowed with lot of indigenous medicinal plants with promising therapeutic potential, which are still under exploitation (Madikizela & McGaw, 2017). Some of these medicinal plants had been claimed to be effective in treating viral infections by traditional healers (Van Wyk *et al*., 2008). However, they are without scientific backing (Mehrbod *et al.,* 2018). Some examples of such medicinal plants include *Artemisia afra, Tabernaemontana ventricosa, Cussonia spicata, Rapanea melanophloeos, Clerodendrum glabrum Artemisia absinthium and Pittisporum viridflorum* (Mehrbod *et al.,* 2018).

Artemisia afra Jacq. ex Willd belongs to the family of Asteraceae. It is one of the oldest indigenous Medicinal plant in Southern Africa including Swaziland, South Africa, Nambia, Zimbabwe and Lesotho (Bora & Sharma, 2011) This plant is commonly referred to as "Africa wormwood" (English), "Wilde als" (Afrikanns), "umhlonyane" (isi Zulu), "Lengana" (BaSotho) (Van Wyk *et al*., 2008). It is traditionally employed against myriad maladies like coughs, fever, chest complaint colds and asthma, nasal blockage, gout, dyspepsia, convulsion, rheumatism, measles, mumps, convulsion, earache, diabetes, wounds, kidney disorder and malaria (Bora & Sharma, 2011). Pharmacological effects including antiviral, antibacterial and anti-inflammatory activities of the plant have also been demonstrated (Mukinda & Syce, 2007). The pharmacological activities of this plant are attributed to its secondary metabolites constituents including riacontane, monoterpenes, scopoletin, quebrachitol, ses-quiterpenementhatriene, 2-methyl butyl isovalerate, myrcene, myrtenal, myrtenol, -β-ocimene, -β-ocime, β-phellandreneMuyima, mentha-1,8-dien-10-ol, cis-p-mentha-1-(7),8-dien-2-ol, trans-p-meth-2-en-1-ol, α-pinene and β-pinene (Liu *et al*., 2009).

Artemisia absinthium Linnaeus. (Asteraceae) is endemic to Eurasia and Northern Africa. It is known as wormwood (United Kingdom), absinthe (France) "umhlonyane" (South Africa) (Bora & Sharma, 2011). In traditional medicine, the plant infusion served as tea to relief labor pain during child birth as well as treat sclerosis and leukaemia (Canadanovic-Brunet *et al.,* 2005). The plant major chemical composition includes; polyphenol, flavonoid, condensed tannin, quercetin-3-O-β-D-glucoside, theγ-muurolene, α-humulene, theα-copaen, theγ-curcumene isorhamnetin-3-O-rhamnoglucoside, and isorhamnetin-3-glucoside, isoquercitrin and quercetin-3-O-rhamnoglucoside

(Msaada *et al*., 2015). These compounds dictate for the plant biological activities including; antimalarial, anti-trypanosomal, hepato-protective antifungal, neuroprotective, acarida, antimicrobial, anthelmintic, insecticidal and antidepressant (Johnson *et al.,* 2020).

Pittosporum viridiflorum Sims belongs to Pittosporaceae family. They are found in tropical and sub- tropical region such as Africa, Asia, New Zealand, Pacific island and Australian. It is commonly known as "Chesswood" (English), "Kasuur" (Afrikanns), "umVusanmu" (isi Zulu), "Kgalahangwe" (SeSotho), and "Umkhwenkwe" (Ama Xhosa) (Madikizela & McGaw, 2017). The plant is extensively used in Africa traditional medicine (ATM) against ailments including circulatory, tuberculosis, malaria, inflammatory, cancer, kidney disorders and sexually transmitted diseases (Nyabayo *et al.,* 2015). The plant's decoctions are also used to treat stomach complaints, abdominal pain and fever. Similarly, the dried or powdered root or bark plant material are used as aphrodisiac (Madikizela & McGaw, 2017). The secondary metabolites including alkaloids, saponin, flavonoids, proanthocyandins and phenol have been reported in *P. viridiflorum* (Otang *et al*., 2012).

Medicinal plant including *Artemisia afra, Artemisia absinthium and Pittisporum viridflorum* are commonly used by Xhosa traditionally healers in the Eastern Cape province of South Africa in treatment of respiratory diseases such as influenza. However, there is paucity on the scientific validation of these plants. Therefore, this study focused on investigating the *in vitro* antiviral, antioxidant and *in vivo* antipyretic of various crude extracts prepared from *Artemisia afra, Artemisia absinthium* and *Pittisporum viridflorum.*

MATERIAL AND METHODS

Chemicals/Reagents

Chemicals and reagents used in this study were of analytical grade and purchased from Sigma Aldrich Company Limited (Steinheim, Germany).

Plants collection and identification

The Medicinal plants (*Artemisia afra* Jacq. ex Willd, *Artemisia absinthium* L *and Pittisporum viridflorum* Sims*)* were collected in March 2018 from Mdanstane (32°57'0"S, 27°46'0"E), Eastern Cape, South Africa. The plants were identified by the Chief Botanist of Department of Botany, University of Zululand. The specimens voucher numbers UZFH1, UZFH2 and UZFH3 of the plants have been deposited at the University's herbarium.

Plants preparation and extraction

The leaves of *Artemisia afra, Artemisia absinthiumin and Pittisporum viridflorum* were respectively plucked and rinsed with clean water to remove dirt (Osunsanmi *et al.,* 2019). Afterward they were air dried at room temperature (25°C) for 48 hours and pulverized using Laboratory grinder (Retsch, ZM 200). Pulverized sample (50 kg) of each plant was separately macerated (1: 5 w/v X 3) with different solvents (aqueous; methanol; and dichloromethane) and incubated on a platform shaker (150 rpm; 25ºC) for 72 hours (Osunsanmi *et al.,* 2019). The solvents were refreshed every 24 h. The crude extracts were filtered through Whatman no 1 filter paper and the methanol and dichloromethane filtrates were concentrated *in vacuo* using Buchi (Model R- 200) rotary evaporator (48 rpm; 40ºC) (Osunsanmi *et al.,* 2019). The aqueous crude extracts were lyophilized with Freeze dryer (Virtis benchtop K). Percentage (%) yields (on dry weight basis) of the various crude extracts were calculated by this formula; % yield = (W_2 X 100)/ W_1. W_2 denoted weight of sample after solvent removal whereas, W_I denoted weight of original sample.

Virus

The virus (A/PR/8/34 (H1N1), A/Sydney/5/97 and B/Jiangsu/10/2003) used in the study were obtained from Institute of Materia Medica (IMM), Chinese Academy of Medical Science Beijing, China. They were propagated in an allantoic sac for 15 days with free pathogen embryonated eggs. The virus stocks were stored at -80°C in the refrigerator until required for use (Wei *et al.,* 2015).

Fluorescence based - Neuraminidase inhibitor

Antiviral activity of the crude extracts was investigated using the fluorescence based neuraminidase inhibitor assay as described by Wei *et al.,* (2015) with slight modification. Diluted virus solution (1:2) in assay buffer consisting of 2-(N-morpholino)ethanesulfonic acid (32.5 mM; pH 6.5), calcium chloride (4 mM; 0.1% NP 40) and bovine serum albumin (0.3 mg/mL) were prepared. Portion of this solution (50 µL) was pipetted into respective cells of 96 well plate containing 2- (4-methylumbelliferyl)-a-d-N-acetylneuraminic acid (MUNANA) (50 µL). The 96 well plate was then incubated (45 minutes; 37°C). Afterward, each portion (50 µL; 400 µg/mL) of crude extracts, tween

20 (negative control), Zanamivir (positive control) were pipetted into respective cells of the 96 well plate, and was further incubated (20 minutes; 37°C). Prepared Stop solution (100 µL; 0.14 M NaOH in 83% ethanol**)** was added to terminate the reaction. Emitted 4-methylumbelliferone was fluorometrically quantified using Biotek SYNERGY HT plate reader at emission (448 nm) and excitation (360 nm) wavelengths. The percentage Neuraminidase inhibitory activity of the crude extracts was determined from the formula; = $[(N_1 – N_2)/N_1] \times 100$. N_1 denotes control whereas N_2 indicates crude extracts treatment. Secondary screening of extracts with higher activities (more than 50%) was carried out in consecutive fivefold serial dilutions to calculate their IC_{50} values of NA inhibitory activities.

Animals preparation for study

Ethical certificate (UZREC 171110-030PGD 018/S76) was issued by the Research Ethic committee of University of Zululand for animal experiments. Male Sprague Dawley rats (200 -250 g) were obtained from the Animal house at the Department of Biochemistry and Microbiology, University of Zululand. International guidelines on handling of animals was adopted (National Institute of Health, 2002). The rats were kept inside wire mesh cage in a controlled room (25°C; 12 h light/12 h dark) with access to regular food and water *ad libitum*.

In Vivo Pyrexia study

The i*n vivo* anti-pyrexia potential of the crude extracts was determined using the method of Gao *et al.,* (2014). Prior to the oral administration of the crude extracts and standard drug, pyrexia (fever) was induced in the rats through subcutaneous injection of yeast (12%; 1 mL/kg body weight). The rats with an increase in temperature of 1.6°C were considered pyrexia. The pyretic rats were divided into eleven groups of four each. Group 1, the negative control received Caboxymethylcellulose, (CMC) the drug vehicle. Group 2 received paracetamol (Panadol) intraperitoneally (40 mg/kg bw; 0.5 mL); the animals in Groups 3-11 each received the respective dose (500 mg/kg body weight; 0.5 mL) intraperitoneally of *Artemisia afra* methanol extract, *Artemisia absinthium* methanol extract, *Pittisporium viridflorium* methanol extract, *Artemisia afra* dichloromethane extract, *Artemisia vulgaris* dichloromethane extract, *Pittisporium viridflorium* dichloromethane extract, *Artemisia afra* aqueous extract, *Artemisia absinthium* aqueous extract, and *Pittisporium viridflorium* aqueous extract. Temperatures of each rats in the respective groups were monitored every 30 minutes' interval until 4 hours of treatments, using rectal thermometer (Checktemp M2512410).

Antioxidant studies

Antioxidant studies for only methanol crude extracts from the plant materials were screened due to limited available reagent for the study.

1 1, -diphenyl-2-picryl hydrazyl (DPPH) scavenging activity

The ability of the methanol crude extracts to scavenge DPPH radical was evaluated using the method reported by Huda-Faujan *et al*. (2009), with slight modification. DPPH (2 mL; 0.1 mM) was thoroughly mixed with crude extracts (2 mL) of concentrations (1, 2, 3, 4 and 5 mg/100 mL Butylated hydroxyl anisole (BHA) and Ascorbic acid served as positive controls. The mixtures were incubated for 60 minutes at 25°C. Absorbance was read at 517 nm using the BioTek SYNERGY HT plate reader. The percentage DPPH radical inhibitory activity of crude extracts were calculated as follows; % DPPH= $[(A_1 – A_2)/A_1] \times 100$. A_1 denotes control whereas A_2 indicates crude extracts treatment.

Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activity of the crude extracts was investigated using Griess IIosovy reactions as described by Govindarajan *et al.* (2005), with slight modification. Different concentrations (as used in the DPPH test above) of crude extracts were pipetted (20 µL) into respective cells of 96 wells plate along with sodium nitroprusside phosphate-buffered saline (30 µL; 0.2 M; pH 6.6). The mixtures were incubated at 25°C for 2 hours. Afterward, sulfanilic acid (0.1 µL; 0.33% in 20% glacial acetic acid) was pipetted into respective cells. It was further incubated for 5 minutes. After completion of diazotization reaction, Naphthyl-ethylenediamine dihydrochloride (0.2 µL, 0.1%) was added to the mixture. The 96 well plate was again incubated (30 minutes; 25°C) and absorbance was read at 540 nm using BioTek SYNERGY HT plate reader. Butylated acid and ascorbic acid served as positive control. The percentage (%) NO scavenging potential of crude extract was calculated using the formula similar to that used for the DPPH activity.

Hydroxyl radical (OH) scavenging

Hydroxyl radical scavenging activity of methanol crude extracts was determined based on hydroxyl radicals generated from Haber Weiss reaction, as described by Payithra & Vadivukkarasi (2015). Crude extracts (0.2 mL) of various concentration (1, 2, 3, 4, 5 mg/100 mL) were mixed with hydrogen peroxide (200 µL: 10 mM) in a capped test tube containing 0.2 mL each of Ethylenediaminetetra-acetic acid (EDTA;10 mM), phosphate buffer (pH 7.4; 0.1 M), 2-Deoxyribose (10 mM) and $FeSO_4$. 7H2O (10 mM). The mixture was then incubated at 37°C for 4 hours. Afterward, trichloroacetic acid (1.0 mL; 2.8%) and tertiary butyl alcohol (1.0 mL; 1%) was pipetted to the respective test tubes and boiled for 10 minutes in a water bath. Butylated acid and trolox served as positive control The mixture was allowed to cool and absorbance read at 520 nm on a BioTek SYNERGY HT plate reader. Percentage hydroxyl (OH) scavenging activity was calculated with the following formula % OH = $[(A_1 – A_2)/A_1] \times 100$. A_1 denotes control whereas A_2 indicates crude extracts treatment.

Superoxide anion scavenging

Superoxide anion scavenging potential of methanol crude extracts was investigated as described by Goh *et al.,* (2010). Crude extracts (2 mL) of various concentration (1, 2, 3, 4 and 5 mg/100 mL) were mixed in a test tube containing 0.02 mL of each copper (II) chloride (6 mM), xanthine oxidase (6 mµ), bovine serum albumin (0.15%), xanthine (3 mM), ethylenediaminteraacetic acid (3 mM) and nitroblue tetrazolium (0.75 mM) respectively. The mixture was incubated (20 minutes; 25°C) and absorbance read at 560 nm on a BioTek SYNERGY HT plate reader. Ascorbic acid and Butylated acid served as positive control. Percentage superoxide anion **s** scavenging activity was calculated as % Superoxide anion = $[(A_1 – A_2)/A_1] \times 100$. A_1 denotes control whereas A_2 indicates crude extracts treatment.

Phytochemical analysis of Plants material (quantitative)

Total flavonoid, phenol and proanthocyanidin content of *Artemisia afra, Artemisia absinthium Pittisporum viridiflorum* were investigated.

Phenolic content

Phenolic content of *Artemisia afra, Artemisia absinthium and Pittisporum* was determined as described by Wolfe *et al.,* (2003). Different concentrations (0.1 mg/mL, 0.2 mg/mL, 0.4 mg/L, 0.6 mg/L, 0.8 mg/L and 1.0 mg/mL) of the plants material were mixed with Folin-Ciocalteau reagent (5 mL; 10%) and sodium carbonate (4 mL; 7.5%) in their respective test tubes. The mixture was vortexed (15 seconds) and incubated (30 minutes; 25°C). Absorbance was read at 765 nm using a BioTek Synergy HT microplate reader. Total phenolic content was expressed as mg/mL of gallic acid equivalent.

Flavonoid content

Flavonoid content of the plant materials was determined using method described by Ordonez *et al.,* (2006). Aluminium chloride in ethanol (5 mL; 2%) was thoroughly mixed with different concentration (0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL and 1 mg/mL) prepared from pulverized plant materials in respective test tubes. The solution was incubated (1 hour; 25°C) until colour changed to yellow. Absorbance was read at 420 nm using BioTek Synergy HT microplate reader. Total flavonoid content was calculated as quercetin equivalent.

Proanthocyanidin content

Proanthocyanidin in the plants was determined following the method of Sun *et al.,* (1998). Plants samples (0.5 mL) of various concentration (0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL and 1 mg/mL) were mixed thoroughly in respective test tubes containing Vallin-methanol solution (3.0 mL; 4%) and concentrated hydrochloric acid (1 mL). The resultant solution was incubated (15 minutes; 25°C). Absorbance was read at 500 nm with the BioTek Synergy HT microplate reader. Total proanthocyanidin contents were expressed as mg/mL of catechin equivalent.

Data analysis

Experiments carried out in this study were triplicated and data expressed as mean ± standard deviation (SD). Post hoc Tukey test and one-way ANOVA were calculated using Graph Pad Prism (version 5.03). Statistical significances were considered at $p<0.05$. The concentration that could affect 5% inhibitory activity (IC_{50}) was calculated using linear regression graph.

RESULTS

Percentage yield of crude extracts

Percentage yield (Table No. 1) of the various crude

extracts from the plants material showed that the**.** aqueous extracts were significantly (p<0.05) higher compared with other solvents (methanol and dichloromethane); and the aqueous crude extract of *Pittisporum viridiflorum* showed the highest percentage yield compared with the other plant materials of the same treatment.

Table No. 1

Plants	Aqueous	Methanol	Dichloromethane
Artemisia afra	7.0 ± 0.09^{a}	3.23 ± 0.04^{d}	4.75 ± 0.02^{c}
Artemisia absinthium	8.6 ± 0.06^{b}	3.38 ± 0.08^{d}	3.81 ± 0.07^{f}
Pittisporum viridiflorum	9.0 ± 0.19^{b}	2.89 ± 0.03^{e}	4.97 ± 0.08^{c}

Percentage yield (dry weight basis) of *Artemisia afra*, *Artemisia absinthium*, *Pittisporum viridiflorum* crude extracts using various solvents treatment. Data represented as mean ± SD. Values with different alphabets (a, b, c, d, e, f) were significance (p<0.05)

Fluorescence based - Neuraminidase inhibitor activity

Table No. 2 depict the percentage neuraminidase inhibition of the crude extracts against the three influenza virus (A/PR/8/34, A/Sydney/5/97 and B/Jiangsu//10/2003). Different degree of efficiency against the tested influenza virus was observed; it is noted that the aqueous extracts (especially, MAA, AAV, and APV) were more efficient than the other extracts and they exhibited activities that were > 40% inhibition against the three tested viruses.

Anti-Pyrexia activity

The effects of the crude extracts of the leaves of *Artemisia afra, Artemisia absinthium, and Pittisporum viridiflorum* on yeast induced pyrexia in rats are presented in Table No. 3. It is apparent that the yeast treatment induced pyrexia in rats as evidenced by the significant increase in temperature (38.3°C – 40.1°C) when compared with prior injection temperature range (36°C – 36.4°C). This hike in temperature prolonged for 30 minutes in-respect of treatments in all the groups. However, after one hour until four hours post injection time frame, all the crude extracts significantly countered the yeast induced fever in the tested rats. In addition to this, all the crude extracts compared favourably with paracetamol, the positive control.

Antioxidant activity

The antioxidant potentials (free radical scavenging) of the methanol extracts of the plants is presented in Table No. 4. MAA showed significant highest antioxidant ability against DPPH (IC_{50}: 3.31 ± 0.14 mg/100 mL), NO scavenging (IC_{50}: 2.09 ± 0.11 mg/ 100 mL) and Superoxide anion (3.71 ± 0.14 mg/100 mL) respectively in comparison with other treated plant materials. However, MAV (IC_{50}: 4.39 ± 0.14 mg/100 mL) showed highest antioxidant activity against hydroxyl radical compared with MAA and MPV.

Table No. 2

Treatment	A/PR/8/34 (H1N1)	A/Sydney/5/97 (H3N2)	B/Jiangsu//10/2003
MAA	49.84 ± 2.73[a]	40.13 ± 3.41 [c]	52.04 ± 2.08[a] (76.11±3.84)
MAV	36.3 ± 4.99[c]	45.48 ± 4.00 [a]	47.06 ± 6.21 [a]
MPV	-29.80 ± 3.56[n]	-36.73 ± 3.31[n]	0.61 ± 1.61 [h]
DAA	28.04 ± 2.43[d]	35.88 ± 6.51 [c]	49.51 ± 2.77 [a]
DAV	-18.73 ± 4.38 [e]	1.64 ± 1.57 [h]	14.59 ± 5.80 [e]
DPV	-31.98 ± 3.06 [n]	-32.61 ± 6.98[n]	-7.86 ± 1.36 [p]
AAA	35.01 ± 5.97[c]	42.87 ± 1.84[a]	46.10 ± 2.22 [a]
AAV	45.34 ± 5.13[a]	55.53 ± 2.88[g] (69.95 ± 4.03)	64.06 ± 4.10[b] (50.05 ± 3.27)
APV	47.58 ± 5.43[a]	57.11 ± 2.61[g] (64.69 ± 4.77)	47.67 ± 2.81 [a]
Zanamivir	89.95 ± 4.03[f] ($4.02 \pm 1.81 \times 10^{-5}$)	96.11 ± 3.84[l] ($1.80 \times 10^{-4} \pm 1.05 \times 10^{-4}$)	75.05 ± 3.27 [k] ($6.47 \times 10^{-5} \pm 0.52 \times 10^{-5}$)

Percentage (%) neuraminidase inhibition of various crude extracts (400 ug/mL). Experiment was triplicated and data represented as mean ± standard deviation (SD). Values with different superscript alphabets were significant ($p<0.05$). IC_{50} (ug/mL) values of the active extracts in parentheses *Artemisia afra* methanol crude extract (MAA), *Artemisia absinthium* imethanol crude extract (MAV) *Pittisporum viridiflorum* methanol crude extract (MPV), *Artemisia afra* dichloromethane crude extract (DAA), *Artemisia absinthium* dichloromethane crude extract (DAV) *Pittisporum viridiflorum*, dichloromethane crude extract (DPV), *Artemisia afra* aqueous crude extract (AAA), *Artemisia absinthium* aqueous crude extract (AAV) *Pittisporum viridiflorum* aqueous crude extract (APV)

Table No. 3

Treatment	Prior injection	Post injection 30 minute	Post injection 1 hour	Post injection 2 hours	Post injection 3 hours	Post injection 4 hours
CMC	36.0 ± 0.84[a]	39.0 ±1.04[b]	39.3 ±2.83[b]	39.4±0.44 [b]	39.6 ±1.72[b]	39.4 ±1.39[b]
Paracetemol	36.4 ±2.14 [a]	40.1 ±1.44[b]	36.5 ±1.36[a]	36.2±0.34[a]	36.1±4.39 [a]	36±.2.66 [a]
MAA	36.4 ±1.34 [a]	38.7±1.25 [b]	36.9 ±1.68 [a]	36.7±3.07 [a]	36.8 ±3.30 [a]	36.7±0.54 [a]
MAV	36.4 ±1.64 [a]	38.7±1.17 [b]	37 ±0.54 [a]	36.8±2.37 [a]	36.8 ±0.99 [a]	36.7±1.74 [a]
MPV	36.3 ±0.35 [a]	39 ±2.88 [b]	37.1±0.64 [a]	36.7±2.69 [a]	36.8 ±0.44 [a]	36.7±1.07 [a]
DAA	36.6 ±2.92 [a]	38.8±0.33 [b]	36.4 ±0.75 [a]	36 ±1.64 [a]	36 ±0.32 [a]	36 ±3.34 [a]
DAV	36.3 ±0.34 [a]	38 ± 0.86 [b]	36.6 ±1.24	36.5 ±1.34	36.4 ±2.71	36.9 ±0.99
DPV	36.3 ±1.77 [a]	38.7±1.36 [b]	36.6 ±0.94 [a]	36.7±2.49 [a]	36.8 ±0.28 [a]	36.9±1.84 [a]
AAA	36.8 ±0.43 [a]	39.3±0.14 [b]	36 ±2.45 [a]	36 ±0.94 [a]	36 ±2.99 [a]	36 ±2.04 [a]
AAV	36.3 ±1.31 [a]	38.9±1.04 [b]	36.4 ±0.94 [a]	36.7±1.66 [a]	36.8±1.84 [a]	36.7±1.84 [a]
APV	36 ±0.55 [a]	38.9±1.94 [b]	36.5 ±2.34 [a]	36.3±2.86 [a]	36.4 ±1.04 [a]	36 ±2.44 [a]

Table No. 4

Treatment	DPPH	Nitric oxide	Hydroxyl Radical	Super Oxide
MAA	3.31 ± 0.14 [b]	2.09 ± 0.11[c]	ND	3.71 ± 0.14[b]
MAV	ND	3.44 ± 0.05 [b]	4.39 ± 0.14 [d]	ND
MPV	ND	3.37 ± 0.75 [b]	ND	ND
BHA	1.68 ± 0.04 [a]	1.27 ± 0.01 [a]	ND	2.15 ± 0.09 [c]
Ascorbic acid	4.25 ± 0.02 [d]	1.54 ± 0.03 [a]	4.53 ± 0.34 [d]	2.49 ± 0.10 [c]
Trolox	ND	ND	4.28 ± 0.26 [d]	ND

The IC_{50} (mg/100 mL) values of *Artemisia afra*, *Artemisia absinthium* and *Pittisporum viridiflorum* methanol crude extracts against DPPH, nitric oxide (NO), hydroxyl radical and super oxide anion radicals. Experiment was triplicated and data represented as mean ± SD Values with different alphabets (a, b, c, d) were significant (p<0.05). *Artemisia afra* methanol crude extract (MAA), *Artemisia absinthium* methanol crude extract (MAV), *Pittisporum viridiflorum* methanol crude extract (MPV), butylated hydroxyanisole (BHA), not determined ND

Phytochemical analysis

Table No. 5 shows some phytochemicals detected in the plants material. All the plants contained appreciable quantities of phenolic compounds, flavonoids, and pro-anthocyanins. However, *Artemisia absinthium* showed the highest significant Pro-anthocyanin content in comparison with other plants material. Whereas Artemisia *afra* showed the least phenolic content with other plants counterparts.

Table No. 5

Plants	Phenolic (mg/mL)	Flavonoids (mg/mL)	Pro-anthocyanin (mg/mL)
Artemisia *afra*	23.7 ± 0.11[a]	26 ± 0.27[a]	329 ± 0.18[c]
Artemisia absinthium	40.15 ± 0.04[b]	24.5 ± 0.16[a]	680.5 ± 0.33[d]
Pittisporum viridiflorum	40.75 ± 0.36[b]	26.5 ± 0. 03[a]	590.3 ± 0.14[e]

Total flavonoid, phenol and proanthocyanidin content of *Artemisia afra*, *Artemisia absinthium*, *Pittisporum viridiflorum*. Experiment was triplicated and data expressed as mean ± SD. Values with different alphabets (a, b, c, d, e, f) indicate significant differences (p<0.05)

DISCUSSION

History reveals that the current COVID-19 pandemic is not a single event. The repeated occurrence of the Ebola indicates that further waves of infection should be expected in the future. There is therefore, an urgent need to develop novel antiviral remedy against failing vaccine and other orthodox antiviral drugs in managing the present and future new strains of respiratory virus infections (Wei *et al.*, 2015). In 2010, WHO suggested the development of more effective strategic treatment to contain viral pandemic, and there is the growing interest in natural and traditional medicines as a source of new products. Medicinal plants have long been recognized as the major healthcare resources by rural dwellers and urban elite in developing countries (Mehrbod *et al.*, 2018).

Virus neuraminidase influences the release of newly synthesised progeny virion from the infected host cells. It also encourages virus replications and spread within the infected cells (Stoner *et al.*, 2010; Wei *et al.*, 2015). Therefore, neuraminidase inhibitors (NAI) become ideal target to halt the virus virulence (Moscona, 2005). In this study, crude extracts from the medicinal plants (*Artemisia afra, Artemisia absinthium and Pittisporum viridflorum*) inhibited the neuraminidase activity of the influenza virus (A/PR/8/34, A/Sydney/5/97 and B/Jiangsu//10/2003) at different threshold (Table No. 2). It is apparent that these crude plants extracts contain active components with promising neuraminidase inhibitor NAI potential (Monto, 2020). Solvents types influences the solubility of phytochemical components from particular plants material based on their polarity potential (Sultana & Ashraf, 2009). The aqueous extracts gave (Table No. 1) the highest percentage yield and they were the most potent of all the extracts (Table No. 2). This could denote that the plant's materials consist of more polar soluble compounds than non-polar compounds, and that active

components could be polar in nature (Gupta *et al.*, 2016). It is worth noting that traditional healers prepare the concoctions for their patients with water. The variation in NAI activity of the crude extracts could be attributed to the varying phytochemicals that the plants possess. Moreover, the neuraminidase mutated genes of virus strains has demonstrated varying NAI susceptibility (Wei *et al.*, 2015). Wei *et al.* (2015), reported on the variation of antiviral activity of *Chongkukjang* crude extracts against A/chicken/Korea/MS96/96 (H9N2) and A/NWS/33 (H1N1) influenza

Hyperpyrexia (≥ 37.8°C) is one the commonest clinical symptoms of viral infections (Chughtai *et al*., 2017). The shift in body core temperature is attributed to the secretion of pyrogens by macrophages and leucocytes exposed to the virus (Plaza *et al*., 2016). Although fever might be beneficial to facilitate host's metabolic activities to undo virus virulences and initiate repairs of damaged tissues, uncontrollable fever however, could escalate hyperpyrexia which is are detrimental to body physiology (Tomiyama *et al.,* 2015)**.** Fever encourages expression of heat shock protein (HSP 70) which further stabilizes the virus on the host (Gao *et al.,* 2014). Likewise, it alters intracellular cholesterol homeostasis that dictate membrane fluidity (Plaza *et al.,* 2016). These processes promote virus entry and its replications. Hence, strategic efforts are required to counter fever in managing viral infections. Over the years, yeast has been used to induce pyrexia which has been described as 'pathogenic fever'; it is accompanied by inflammatory reaction caused by proteins of yeast or capsular polysaccharides (Gao *et al.,* 2014). All the crude extracts treatments in this study showed (Table No. 3) appreciable anti-pyretic effects in the pyretic rats. The added advantage is that traditional healers could be using these plants to treat both the virus and the accompanied fever. The antipyretic activity of crude extracts of medicinal plants on yeast induced pyrexia using animal models have been reported (Nethengwe *et al*., 2012; Khan *et al.,* 2015). Clinically, paracetamol (the first choice in the treatment of fever) inhibits cyclooxygenase-2 (COX_2) that reduce activity of prostaglandin E2 (PGE2), which activity sustains fever (Twycross *et al.,* 2013). Yet paracetamol has been associated with side effects including; acute liver necrosis, bladder carcinoma etc. In addition, exposure to paracetamol overtime could increase risk of developing asthma (Gao *et al.,* 2014). In view of these, natural antipyretic agents like the crude extracts in this study could be the preferable remedy. Antipyretic potential of the crude extracts could be attributed to reduction of prostaglandin E biosynthesis. In previous study, some crude extracts displayed antipyretic activities by decreasing level of interleukin 8 and prostaglandin E on yeast induced pyrexia (Liu *et al*., 2017b). Additionally, the plants chemical constituents including phenol, flavonoid and pro-anthocyanin could also contributed to the antipyretic activity (Aouey *et al*., 2016).

Emerging evidence shows that oxidative stress is a major contributing factors to infectious diseases**,** like influenza, *hepatitis C virus* (HCV), *hepatitis B virus* (HBV) and *Herpes Simplex Virus* (HSV) (Mileva, 2016). Reactive oxygen species (ROS) produced during oxidative stress are by products of mitochondria metabolism such as singlet oxygen (O_2), hydrogen peroxide, super oxide radical, hydroxyl radical and other highly reactive molecules (Afolayan *et al.,* 2013). This makes host infected cells in a chronic oxidative stress state which accelerate deterioration of the diseases (Liu *et al.,* 2017a). The antioxidant status of these plants (Table No. 4) could be an added advantage to them being used in the management of virus associated respiratory diseases. The antioxidant potential of various medicinal plants has been screened and reported (Nethengwe *et al*., 2012; Afolayan *et al.,* 2013). The antioxidant status (especially the free radical scavenging) of plants has been attributed to the phenolic compounds, the flavonoids and pro-anthocyanin content (Table No. 5) of the plants (Uzunuigbe *et al.,* 2019).

CONCLUSION

This study has provided an assessment of the three medicinal plants (*Artemisia afra*, *Artemisia absinthium* and *Pittisporum viridiflorum*) as promising NAI agents against respiratory infection tract virus strains. The observed activities of the plants' extracts were not as potent as Zanamivir, but it is recognized that the plants extracts were crude preparations. In addition, the plants also displayed antioxidant and antipyretic activities at different threshold. This study however, establishes the scientific validation of the use of these plants in folk medicine and provides good backdrop for the extraction and characterization of the active components (possibly novel) in the plants.

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REFERENCES

Afolayan AJ, Sharaibi OJ, Kazeem MI. 2013. Phytochemical analysis and *in vitro* antioxidant activity of *Nymphaea lotus* L. **Int Res J Pharm** 9: 297 - 304. **https://doi.org/10.3923/ijp.2013.297.304**

Aouey B, Samet AM, Fetoui H, Simmonds MSJ, Bouaziz M. 2016. Anti-oxidant, anti-inflammatory, analgesic and antipyretic activities of grapevine leaf extract (*Vitis vinifera*) in mice and identification of its active constituents by LC-MS/MS analyses. **Biomed Pharmacother** 84: 1088 - 1098. **https://doi.org/10.1016/j.biopha.2016.10.033**

Bora KS, Sharma A. 2011. The genus *Artemisia*: a comprehensive review. **Pharm Biol** 49: 101 - 109.

Canadanovic-Brunet M, Djilas SM, Cetkovic GS, Tumbas VT. 2005. Free-radical scavenging activity of wormwood (*Artemisia absinthium* L) extracts. **J Sci Food Agric** 85: 265 - 272. **https://doi.org/10.1002/jsfa.1950**

Chughtai A, Wang Q, Dung TC, Macintyre CR. 2017. The presence of fever in adults with influenza and other viral respiratory infections. **Epidemiol Infect** 145: 148 - 155. **https://doi.org/10.1017/s0950268816002181**

Gao X, Gup M, Li Q, Peug L, Liu H, Zhang L, Bai X, Wang Y, Li J, Cai C. 2014. Plasma metabolomic profiling to reveal antipyretic mechanism of *Shuang-Huang-Lian* injection on yeast-induced pyrexia rats. **Plos One** 9: 23 - 26. **https://doi.org/10.1371/journal.pone.0100017**

Goh S, Loh S, Yusoff FM. 2010. A comparison of antioxidant properties and total phenolic content in a Diatom, *Chaetoceros species* and green microalaga, *Nanochloropsis species.* **J Agric Sci** 2: 123 - 130.

Govindarajan R, Vijayakumar M, Pushpangadan P. 2005.Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. **J Ethnopharmacol** 99: 165 - 178. **https://doi.org/10.1016/j.jep.2005.02.035**

Gupta J. 2016. Comparison of different solvents for phytochemical extraction potential from *Datura metel* plant leaves. **Int J Biol Chem** 11: 17 - 22. **https://doi.org/10.3923/ijbc.2017.17.22**

Huda-Faujan N, Noriham A, Norrakiah AS, Babji AS. 2009. Antioxidant activity of plants methanolic extracts containing phenolic compounds. **Afr J Biotechnol** 8: 484 - 489.

Johnson S, Rodriguez D, Allred K. 2020. A Systematic Review of Essential Oils and the Endocannabinoid System: A Connection Worthy of Further Exploration. **Evid Based Complementary Altern Med** 2020: 1 - 13. **https://doi.org/10.1155/2020/8035301**

Kalyani P, Kamaruz Z. 2013. Antiviral activity of some Indian medicinal herbs. **Ind J Pharm Pract** 6: 13 - 14.

Khan MA, Wadud A, Tariq M, Nazamuddin MD. 2015. Antipyretic activity of crude and aqueous extract of *Gule ghaafis* (*Gentiana olivieri* griseb) on yeast induced pyrexia in animal model. **Int J Pharm Sci Res 6**: 3528 - 3533.

Liu C, Su H, Wan H, Qin Q, Wu X, Kong X, Lin N. 2017b. Forsythoside A exerts antipyretic effect on yeast-induced pyrexia mice via inhibiting transient receptor potential vanilloid 1 function. **Int J Biol Sci** 13: 65 - 75. **https://doi.org/10.7150/ijbs.18045**

Liu M, Chen F, Liu T, Chen F, Liu S, Yang S. 2017a. The role of oxidative stress in influenza virus infection**.** **Microbes Infect** 19: 580 - 586. **https://doi.org/10.1016/j.micinf.2017.08.008**

Liu NQ, Van der Kooy F, Verporate R. 2009. *Artemisia afra*: A potential flagship for African medicinal plant. **South Afric J Bot** 75: 185 - 195. **https://doi.org/10.1016/j.sajb.2008.11.001**

Madikizela B, McGaw LJ. 2017. *Pittosporum Viridiflorum* Sims (Pittosporaceae): A review on a useful medicinal plant native to South Africa. **J Ethnopharmacol** 205: 217 - 230. **https://doi.org/10.1016/j.jep.2017.05.005**

Mehrbod P, Abdalla MA, Njova EM, Ahmed AS, Fotouhi F, Farahmand B, Gado DA, Tabatabaian M, Fasanmi OG, Eloff JN, McGaw LJ, Fasina FO. 2018. South African medicinal plant extracts active against influenza A virus. **BMC Complement Altern Med** 18: 112. **https://doi.org/10.1186/s12906-018-2184-y**

Mileva M. 2016. Oxidative stress as a target for medication of influenza virus infection. **Acta Microbiol Bulg** 32: 3 - 7.

Monto AS. 2020. Effectiveness of the neuraminidase inhibitors: The supporting evidence increases. **J Infect Dis** 221: 340 - 342.

Moscona A. 2005. Neuraminidase inhibitors for influenza. **N Engl J Med** 353: 1363 - 1373.

https://doi.org/10.1056/nejmra050740

Msaada K, Salem N, Bachrouch O, Bousselmi S, Tammar S, Alfaify A, Sane KA, Ammar WB, Azeiz S, Brahim AH, Hammami M, Selmi S, Limam F, Marzouk B. 2015. Chemical composition and antioxidant and antimicrobial activities of Wormwood (*Artemisia absinthium* L.) essential oils and phenolics. **J Chem** 2015: 1 - 12. **https://doi.org/10.1155/2015/804658**

Mukinda JT, Syce JA. 2007. Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. **J Ethnopharmacol** 112: 138 - 144. **https://doi.org/10.1016/j.jep.2007.02.011**

National Institute of Health. 2002. **Public health service policy on human care and use of laboratory animals**. USA.

Nethengwe MF, Opoku AR, Dludla PV, Madida KT, Shonhai A, Smith P, Singh M. 2012. Larvicidal, antipyretic and antiplasmodial activity of some Zulu medicinal plants. **J Med Plant Res** 6: 1255 - 1262.

Nyabayo CT, Matasyoh JC, Mwendia C. 2015. *In vitro* assessment of pentacyclic triterpenoids from leaves of *Pittosporum viridiflorum* against east coast fever vector *Rhipicephalus appendiculatus*. **Am J Chem A** 2: 95 - 100.

Ogbole OO, Akinleye TE, Segun PA, Faleye TC, Adeniji AJ. 2018. *In vitro* antiviral activity of twenty-seven medicinal plant extracts from Southwest Nigeria against three serotypes of Echoviruses. **Virol J** 15: article 110. **https://doi.org/10.1186/s12985-018-1022-7**

Ordonez AAL, Gomez JD, Vattuone MA, Isla MI. 2006. Antioxidant activities of *Sechium edule* (Jacq). **Food Chem** 97: 452 - 458. **https://doi.org/10.1016/j.foodchem.2005.05.024**

Osunsanmi, FO, Zharare GE, Opoku· AR. 2019. Phytochemical constituents and antioxidant potential of crude extracts from *Lippia Javanica* (Burm.f.) Spreng leaves. **Pharmacogn J** 11: 24 - 27. **https://doi.org/10.5530/pj.2019.11.128**

Otang, WM, Grierson DS, Ndip RN. 2012. Phytochemical studies and antioxidant activity of two South African medicinal plants traditionally used for the management of opportunistic fungal infections in HIV/AIDS patients. **BMC Complement Altern Med** 12: 43 - 51. **https://doi.org/10.1186/1472-6882-12-43**

Payithra K, Vadivukkarasi S. 2015. Evaluation of free radical scavenging activity of various extracts of leaves from *Kedrostis foetidissima* (Jacq.) Cogn. **Food Sci Hum Well** 4: 42 - 46. **https://doi.org/10.1016/j.fshw.2015.02.001**

Plaza JJG, Hulak N, Zhumadilov Z, Akilzhanova A. 2016. Fever as an important resource for infectious diseases research. **Intractable Rare Dis Res** 5: 97 - 102. **https://doi.org/10.5582/irdr.2016.01009**

Stoner TD, Krauss S, DuBois RM, Negovetich NJ, Stallknecht DE, Senne DA, Gramer, MR, Swafford S, DeLiberto T, Govorkova EA, Webster RG. 2010. Antiviral susceptibility of avian and swine influenza virus of the N1 neuraminidase subtype. **J Virol** 84: 9800 - 9809. **https://doi.org/10.1128/jvi.00296-10**

Sultana A, Ashraf M. 2009. Effects of extraction solvent technique on the antioxidant of selected medicinal plants extracts. **Molecules** 14: 2167 - 2180. **https://doi.org/10.3390/molecules14062167**

Sun B, Ricardo-da-Silva JM, Spranger I. 1998. Critical factors of vanillin assay for catechins and proanthocyanidins. **J Agric Food Chem** 46: 4267 - 4274. **https://doi.org/10.1021/jf980366j**

Tomiyama C, Watanabe M, Honma T, Inada A, Hayakawa T, Ryufuku M, Abo T. 2015. The effect of repetitive mild hyperthermia on body temperature, the autonomic nervous system, and innate and adaptive immunity. **Biomed Res** 36: 135 - 142. **https://doi.org/10.2220/biomedres.36.135**

Twycross A, Finley GA. 2013. Children's and parents' perceptions of postoperative pain management: A mixed methods study. **J Clin Nurs** 22: 3095 - 3108. **https://doi.org/10.1111/jocn.12152**

Uzunuigbe EO, Osunsanmi FO, Mosa RA, Opoku AR, Kappo AP. 2019. Phytochemical constituents and antioxidant activities of crude extracts from *Acacia Senegal* leaf extracts. **Pharmacogn J** 11: 1409 - 1414. **https://doi.org/10.5530/pj.2019.11.218**

Van-Wyk BE. 2008. A broad review of commercially important Southern African medicinal plants. **J Ethnopharmacol** 119: 342 - 355. **https://doi.org/10.1016/j.jep.2008.05.029**

Wei B, Cha S, Kang M, Kim YJ, Cho CW, Rhee YK, Hong HD, Jang HK. 2015. Antiviral activity of *Chongkukjang* extracts against influenza A virus *in vitro* and *in vivo*. **J Ethnic Foods** 2: 47 - 51. **https://doi.org/10.1016/j.jef.2015.04.001**

Wolfe K, Wu X, Liu RH. 2003. Antioxidant activity of apple peels. **J Agric Food Chem** 51: 609 - 614. **https://doi.org/10.1021/jf020782a**