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Biological activities of crude leaf extract and fractions of *Scutellaria sibthorpii* (Benth.) Halácsy: An endemic plant of North-Cyprus

Cagin Korkmazer and Emmanuel Mshelia Halilu
Cyprus International University, Faculty of Pharmacy, Nicosia-Northern Cyprus, Mersin 10, Turkey

Abstract: *Scutellaria sibthorpii* is used in treatment of bacterial infections, pains and inflammations. The leaf was extracted by maceration and then partitioned with hexane, ethyl acetate and n-butanol. The extract was screened for phytochemicals. The antioxidant activity was assayed using DPPH, H_2O_2 and β-carotene. The total phenolic and flavonoid contents were estimated using Folin-Ciocalteu's and $AlCl_3$. The crude extract/fractions were tested on *E. coli*, *S. typhi*, *B. subtilis* and *S. aureus* using agar disk diffusion. Lorke's method was used to determine the LD_{50}. The analgesic activity was determined using ethanoic acid-induced writhing, hotplate and formalin-induced nociception. Anti-inflammatory activity was investigated by means of formalin-induced acute inflammation. The antipyretic activity was studied using Brewer's yeast. The ethyl acetate fraction (EtFSs) had IC_{50} of 0.4352mg/mL in the H_2O_2 and IC_{50} of 0.00014mg/mL. The MIC of EtFSs against *S. aureus* was 1.25mg/mL and 2.5mg/mL on *S. typhi*. LD_{50} of the CrESs/fractions were greater than 5000mg/Kg. The analgesic and anti-inflammatory activities were higher in EtFSs when compared to piroxicam. The fractions decrease the rectal temperature of the rats in the same way as paracetamol at 200mg/Kg. This research has for the first time validated the used of *Scutellaria sibthorpii* traditionally as analgesic, anti-inflammatory and antipyretic.

Keywords: Analgesic, antiinflammatory, antioxidant, antipyretic, antibacterial and *Scutellaria sibthorpii.*

INTRODUCTION

Plants and phytochemicals obtained from them have been used as therapeutic agents and useful drugs have been developed from them (Martemucci *et al*., 2022). Reactive oxygen species (ROS) are generated in human body system due to side reactions and are in constant circulation. The free radicals are eliminated from the body by natural antioxidant processes but if the mechanisms are altered, they accumulate and can cause serious diseases (Martemucci *et al*., 2022; Chaudhary *et al*., 2023). Phyto-antioxidants perform a very important function in the body's defence against oxidants (Martemucci *et al*., 2022). The mitochondria are the main source of endogenous cellular ROS and its excessive generation may lead to the damage of DNA, proteins and lipids. Such damages have been associated with the development of diabetes, tumors, inflammatory, respiratory, cardiovascular and digestive tract diseases (Martemucci *et al*., 2022).

In recent years, antimicrobial resistance (AMR) has been reported by the WHO as a global public health challenge facing humanity (Walsh *et al*., 2023). Globally, it has been forecast that 10 million deaths due to AMR per year may occur by the year 2050 (Tang *et al*., 2023). The contributing factor to the rise in AMR is attributed to overuse and misuse of antibiotics (Tang *et al*., 2023).

Pain disorders and inflammations have been treated with plants as exemplified by *Papaver somniferum* (morphine) and *Salix alba* (aspirin) (Halilu *et al*., 2020). Steroids and non-steroids drugs are currently used in management of pains and inflammations. These medicines are characterized by undesirable adverse reactions (Halilu *et al*., 2020).

Hyperpyrexia is a complex physiological response to an infection and tissue damage. A person is regarded as having fever when the body temperature rises above 37°C and about 75% of patients suffering from illness tends to feel fever (Tegegne and Alehegn, 2023). *Scutellaria sibthorpii* (Benth.) Halácsy (Lamiaceae) is an endemic plant of Cyprus and it is found in the coastal areas around the mountainous in the northern part of the island (Yildiz and Gücel, 2006; Mehrukh *et al*., 2022). The Lamiaceae consists of plants with medicinal values due to their volatile oils, iridoids, ecdysteroids, steroids, flavonoids, anthocyanins, phenylethanoids and caffeoyl esters glycosides (Jamal *et al*., 2018; Çalis and Başer, 2021; Mehrukh *et al*., 2022). *Scutellaria* species have been used as herbal remedies for chemotherapies, hepatoprotection, antiinflammation, antiviral, neuro-protective, intestinal disorders, asthma, neurological and cardiovascular ailments and infectious diseases (Zehravi *et al*., 2022; Jamal *et al*., 2018). Recent studies on the biological activities of *Scutellaria sibthorpii* revealed antioxidant activity which was attributed to the phenolics (Gizem *et al*., 2022). This research has been designed to investigate some chemical and biological activities of *Scutellaria sibthorpii* leaf extract/fractions with the view of validating the uses of the plant in management of pains, inflammations, fever, microbial infections and diseases whose causes are related to free radicals.

**Corresponding author:* e-mail: emshelia2002@gmail.com

MATERIALS AND METHODS

Plant Collection, Identification and preparation

Scutellaria sibthorpii was obtained from the wild by Çağın Korkmazer at the flowering stage on the 28th of May, 2022 from the steep cliffs of the Alevkayası Mountain. The plant identification and authentication was done by Dr. H. E.Mshelia. A voucher specimen with number: CIU/PHAR/LAMI/005 was made and then archived in the Herbarium of the Faculty of Pharmacy, Cyprus International University, for reference purpose. The leaves were shade dried for 3 weeks and was powdered using a blender. The powder (45.118g) was stored until it was required for usage.

Plant extraction

The extraction was done by maceration using 120g of powder plant in 800mL of a mixture of methanol/water (80:20) for 24 hours and then filtered. The marc was washed with 1200mL of the aqueous methanol. A total of 1500 mL of the fitrate was obtained and was concentrated by controlled rotary evaporation to elimate the methanol at 50°C bath temperature, 34°C vapour temperature and 50 rpm.

Fractionation of crude extract

The crude extract (220mL, Brownish in colour) was sucessively fractionated in a separating funnel according to increasing polarity of the solvents starting with hexane (750 mL). The residual water extract was fractionated using ethyl acetate (750mL) and finally the residual water extract was fractionated using 250mL of n-butanol. The successive fractions were air dried in oven at 25°C to produce hexane (HFSs), ethyl acetate (EtFSs) and n-butanol fractions (BFSs). The percentage yields of each fraction were determined.

Qualitative detection of phytochemicals

The CrESs and EtFSs were screened for the precence of flavonoids, saponins, steroids/triterpenoids and tannins as outlined by Halilu *et al*. (2008).

Estimation of total phenolic content (TPC) and total flavonoid content (TFC)

The estimations of TPC and TFC of CrESs and EtFSs were conducted according to the procedures of Sushant *et al*., 2019; Aiyegoro and Okoh, 2010.

Determination of free radical scavenging activity

The evaluations in three models were done on CrESs and the fractions using DPPH assay (Halilu *et al*., 2017); Hydrogen peroxide assay (Sasikumar and Pavithra, 2015) and Beta-Carotene assay (Miraliakbari and Shahidi 2008).

Antibacterial activity

Test microorganisms, preparation of media and determination of zone inhibition

The protocol described by Emmanuel *et al*. (2023) was followed. In this assay, clinical isolates of *E. coli, S. typhi*, *B. subtilis* and *S. aureus* were obtained at Microbiology Laboratory, Cyprus International University. The agar was prepared as specified by the producer. The zone of inhibition of CrESs and fractions were determined using agar disk diffusion method.

Determination of minimum inhibition concentration (MIC) of EtFSs

The EtFSs demonstrated a better activity in the sensitivity test and was subjected to MIC using the LB broth in a 96 well plates. 100µL of 20mg/ml of EtFSs was introduced to the first well and five-fold serial dilution was done. This was repeated for all the 96 wells and 2µL of *S. aureus and S. typhi* were transferred into the wells and were allowed to incubate for the period of 24h at 37˚C. The 96 wells were read using the Elisa spectrophotometer at 630 mn. The MIC was obtained from the concentration which completely inhibited the bacterial growth.

Animal studies

Experimental animals

Male and female mice (17-23g) were sourced from the animal farm of the Faculty of Pharmaceutical Sciences, ABU, Zaria and were transported to Usmanu Danfodiyo University, Sokoto. The mice were stabilized for 14 days prior to the starting of the investigation. Standard chow and H_2O were made available *ad libitium*. The condition of the house was kept at 25±2ºC at 12h day/night cycles with the aid of air condition.

Determination of LD_{50}

The investigation was done in mice by modification of Lorke's method (1983). Male and female mice with weight of 17-23 g were used. The CrESs and fractions were administered intraperitoneally. The mice were observed for 24 hours. The LD_{50} was measured from the highest and lowest doses that the mice survive or died respectively. The LD_{50} was determined as follows:

$LD_{50} = \sqrt{(D_o \times D_{100})}$

Where D_o = Lowest dose that kills the mice; D_{100} = Highest dose that the mice survive.

Analgesic activity

Acetic acid (ethanoic acid) induced writhing

The method of Koster *et al*. (1959) was used. Thirty (30) mature albino mice were categorized into 5 groups having 6 mice each. Group one, two and three were administered 50, 100 and 200mg/kg of CrESs/fractions. After the treatments, the percentage inhibition of constrictions was determined.

Analgesic study (Hotplate method)

The EtFSs produced better response in the ethanoic acid induced pain and was further investigated for analgesia. Male and female mice were subjected to screening by putting them one at a time on the hot plate (55ºC). The mice that failed to lick the hind paw or jump within 15

seconds were removed from the study (Eugene *et al*., 2022). Thereafter, the qualified animals were grouped into five (5) consisting of 6 mice. Groups 1, 2, and 3 were injected with 50, 100 and 200 mg/kg *i.p.* of EtFSs. Group 4 was administered 10 mL/kg *i.p.* of distilled water (negative control) and group 5 was given piroxicam 10 mg/kg (reference drug). The response time of each mouse was noted and the percentage protection was calculated.

$$\text{Protection against thermal}\,(\%) = \frac{\text{Test mean - control mean}}{\text{Control mean}} \times 100$$

Formalin induced pain

The assay was done according to the procedure of Duduisson and Dennis (1977). The groups 1, 2 and 3 were administered 50, 100 and 200 mg/kg *i.p.* of EtFSs. The negative control (group 4) was administered 10 mL/kg *i.p.* of distilled water and the positive control (group 5) was administered with piroxicam 10 mg/kg.

Anti-inflammatory studies (Formalin induced)

The EtFSs was used for the investigation of formalin (2.5%) induced inflammation as described by Winter *et al*. (1962).

Antipyretic activity

The brewer's yeast induced pyrexia as described by Eugene *et al*. (2022) was used to ascertain the antipyretic activity of EtFSs.

Ethical approval

The study was approved by the Animal Research Ethical Committee, Usmanu Danfodiyo University, Sokoto. An ethical clearance with number: PTAC/Ss(HMe)/OT/60-23 was issued. The care and handling of the animals were done according to the established public health guidelines on Guide for Care and Use of Laboratory Animals (2011).

STATISTICAL ANALYSIS

The research data were documented as mean ± SEM. The statistical analysis was executed on GraphPad prism 8. One way analysis of variance (ANOVA) and Dunnet's Multiple comparisons test were applied to compare the means of test and control groups. Differences were considered significant at $p<0.05$.

RESULTS

Extraction

The crude extract appeared as crystalline brownish solid after concentration and drying. table 1 showed the mass and percentage yields of the fractions.

Qualitative screening of phytochemicals

The screening conducted on CrESs and EtFSs uncovered the presence of saponins, tannins, flavonoids and triterpenoids/steroids. Tannin was not detected in the EtFSs.

Table 1: Fractions mass and percentage yield

Solvent	Mass (g)	% Yield
Hexane (HFSs)	1.248	1.04
Ethyl acetate (EtFSs)	7.968	6.64
n-butanol (BFSs)	15.396	12.83

Table 2: TPC and TFC of CrESs and EtFSs

Sample	TPC (mg/GAEmg)	TFC (mg/QEmg)
CrESs	5.7351	0.4416
EtFSs	5.1587	0.3734

GAE=Gallic acid equivalence/mg extract; QE=Quercetin Equivalence/ mg extract

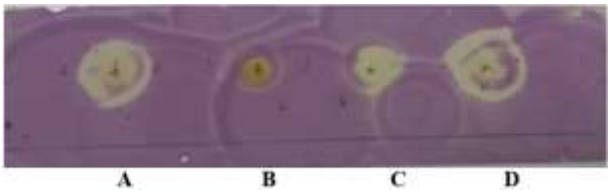

Plate 1 (A, B, C & D): Qualitative screening for Antioxidant activity using DPPH

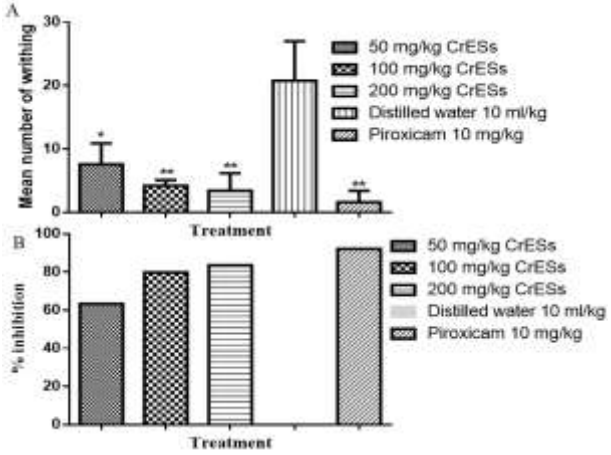

Fig. 1: CrESs on ethanoic acid-induced writhing in mice (A) mean number of writhing (B) percentage inhibition. Values represent mean ± SEM; *p<0.05; **p<0.01 as compared with the control

TPC and TFC of CrESs and EtFSs

The TPC were determined from a standard curved obtained by plotting the various absorbance of gallic acid at concentration of 0.03125-0.5mg/mL (table 2). The TFC were obtained from a standard curved obtained by plotting the absorbances of quercetin at concentration of 0.03125-0.5mg/mL (table 2). The result showed that CrESs had both higher phenolic and flavonoid contents than EtFSs (table 2).

Antioxidant activity

Qualitative screening of free radical scavenging compounds using DPPH

The TLC plate is used as a rapid method for detection of phytochemicals with free radical scavenging activity. These compounds were detected in the CrESs (A), HFSs (B), EtFSs (C) and BFSs (D). The CrESs, HFSs EtFSs

and BFSs demonstrated different degrees of radical scavenging Plates 1: (A, B C and D).

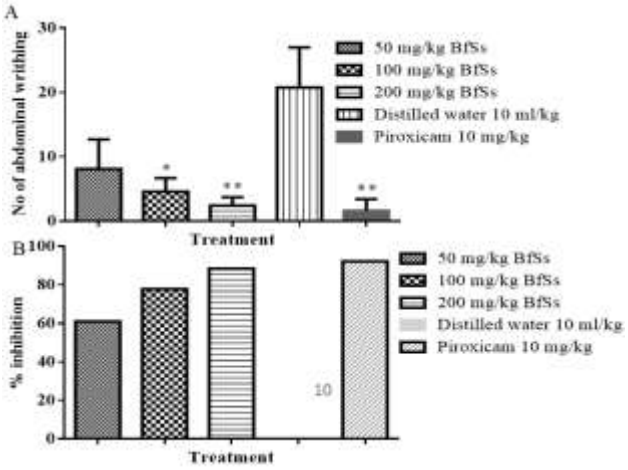

Fig. 2: Effect of BfSs on ethanoic acid-induced writhing in mice (A) mean number of writhing (B) percentage inhibition. Values represent mean ± SEM; *p<0.05; **p<0.01 as compared with the control

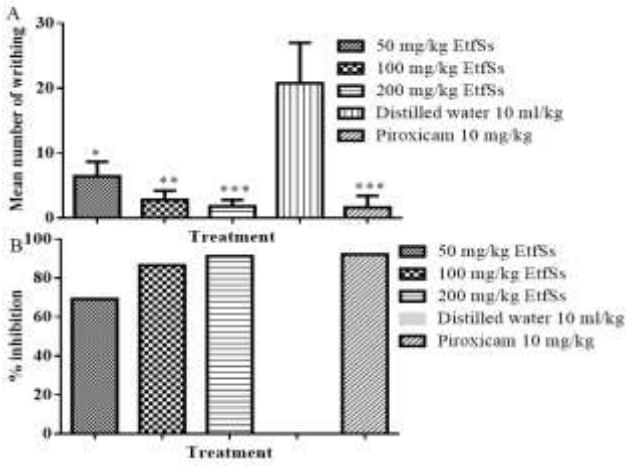

Fig. 3: Effect of EtfSs on ethanoic acid-induced writhing in mice (A) mean number of writhing (B) percentage inhibition.

Values are mean ± SEM; *p<0.05; **p<0.01; ***p<0.001 as compared with the control.

Antibacterial activity

Antibacterial studies of the crude methanol extract

The CrESs inhibited the growth of the bacteria and produced inhibition zones between 7.0mm to 9.7mm (table 4). The HFSs did not show activity on the bacteria. The EtFSs and BFSs demonstrated activity on *S. aureus* with a zone of inhibition of growth of 9mm and 7 mm respectively. Furthermore, the EtFSs inhibited the growth of *S. typhi* with a zone of 7.0mm. The MIC of EtFSs on *S. aureus* was 1.25mg/mL and on *S. typhi* was 2.5mg/mL.

Quantitative antioxidant ssays

The antioxidant activity of CrESs and fractions as tested in various models have been expressed interms of the IC_{50} (table 3).

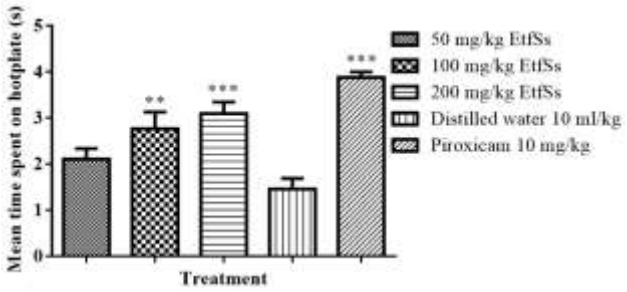

Fig. 4: Effect of EtfSs on hotplate-induced pain in mice.

Values represent mean ± SEM; *p<0.05; **p<0.01; ***p<0.001 as compared with the control

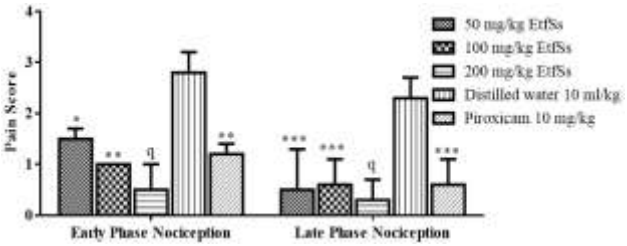

Fig. 5: Effect of EtFSs on formalin-induced pain in rats.

Values represent mean ±SEM; *p<0.05; **p<0.01; ***p<0.001; q p<0.0001 as compared with the control.

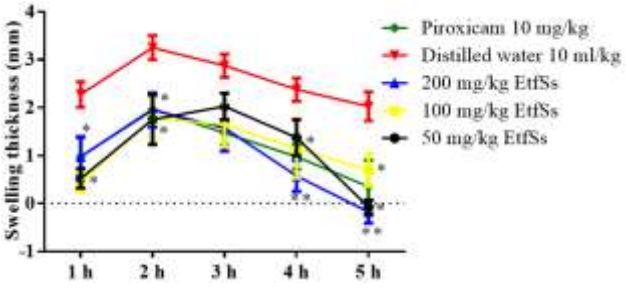

Fig. 6: Effect of EtFSs on formalin-induced acute inflammation.

Values represent mean ± SEM; *p<0.05; **p<0.01

Acute toxicity study

The study in rats revealed that the CrESs/fractions were not toxic since no mortality was recorded within the phase 1 and phase 2 of Lorke's acute toxicity testing (table 5). Although, signs and symptoms of toxicity were observed (table 6). The LD_{50} was found to be ≥ 5000mg/kg.

Analgesic study

Acetic Acid (ethanoic acid) Induced Writhing

The CrESs exerted analgesia at graded dosages against ethanoic acid-induced abdominal constrictions in mice which was significant at $p<0.05$. The inhibition activity was potent at 200mg/Kg (83.57%) which is close to piroxicam (standard drug) 10 mg/kg (92.24%). This result indicated that CrESs exhibited dose-dependent inhibitory activity (fig. 1).

Table 3: IC_{50} of crude extract and fractions

Assays/model	IC_{50} (mg/mL)				
	CrESs	HFSs	EtFSs	BFSs	Ascorbic acid
DPPH	17.26	2.944	6.57	6.57	2.944
H_2O_2	1.234	1.026	0.4352	26.81	9.29
B-carotene	1.124	1.494	0.00014	29.41	2.85

Table 4: Zone of inhibition of the CrESs/fractions at 20 mg/ml in (mm)

Organisms	CrESs	HFSs	EtFSs	BFSs	Gentamicin	Distilled Water
E. coli	8.7	0	0	0	19.3	0
S. typhi	7.0	0	7.0	0	19.7	0
S. aureus	9.7	0	9.0	7.0	19.3	0
B. subtilis	7.0	0	0	0	19.7	0

Table 5: Acute toxicity effects of crude extract and fractions

	Phase 1					
	Dose (mg/kg)	10	100	1000	n	mortality
Test samples						
CrESs					3	0/3
HFSs					3	0/3
EtFSs					3	0/3
BFSs					3	0/3
	Phase 2					
CrESs					1	0/1
HFSs					1	0/1
EtFSs					1	0/1
BFSs					1	0/1

n= number of animals; LD_{50} ≥5000mg/kg

Table 6: Toxicity signs and symptoms

Toxicity signs/symptoms	CrESs	HFSs	EtFSs	BFSs
Calmness	+	-	-	-
Heavy Breathing	+	+	+	+
Bulging of the pulp	+	+	-	+
Licking/scratching of the mouth,	+	-	+	+
Erection of the Pinna's of the ears and furs	+	+	+	+
Restlessness	-	+	+	+
Dark or cherry red colorations of the eye	-	+	+	+
Loss of corneal reflex	-	-	+	+

+ = Presence of sign/symptom of toxicity; - Absence of sign/symptom of toxicity

Table 7: Effect of EtFSs on antipyretic activity

Treatment	Dose (mg/kg)	1 h	2 h	3 h	4 h	5 h
Distilled water	10 mL/kg	1.2±0.15	1.4±0.15	1.7±0.38	0.9±0.43	1.3±0.08
EtFSs	50	0.7±0.16	0.5±0.23	0.3±0.13	0.1±0.56	-0.5±0.28*
EtFSs	100	0.0±0.10	-0.2±0.12	-0.8±0.23*	-1.0±0.30	-1.6±0.31**
EtFSs	200	0.0±0.48	-0.3±0.46	-0.8±0.15**	-1.3±0.60*	-2.0±0.50***
Paracetamol	150	0.9±0.26*	0.4±0.45**	-0.1±0.54***	-0.7±0.21***	-1.1±0.47***

Values represent mean ±SEM. (n=5) *p<0.05; **P<0.01; *** p<0.0001 significant interaction. Dunnet's Multiple comparisons test.

The BFSs and EtFSs exerted similar activity as CrESs. The highest inhibition was observed at 200mg/Kg (88.43%) which is near to piroxicam10mg/kg (92.24%). This outcome indicated that the BFSs demonstrated dose-dependent inhibitory activity (fig. 2). The highest inhibitory effect of EtFSs was observed at 200mg/Kg (91.32%) which is comparable to piroxicam 10mg/kg (92.24%). This outcome indicated that the EtFSs exhibited dose-dependent inhibitory activity (fig. 3).

Formalin induced nociception

The EtFSs inhibited the early phase (neurogenic pain) of the formalin-induced pain in rats at the dose 50, 100 and 200 mg/kg in a dose-dependent pattern. The percentage inhibitions obtained were 46.4, 64.3 and 81.9% for 50, 100 and 200mg/Kg of the ethyl acetate fraction respectively. The EtFSs produced a significant inhibition of the late phase at the dose of 50, 100 and 200mg/kg and the corresponding percentage inhibitions were 78.0, 74.0 and 86.8% respectively. On comparing the EtFSs with the positive control (piroxicam 10mg/kg), the EtFSs at 100 and 200 mg/kg showed a higher percentage inhibition in both the early phase and the late phase than piroxicam 10 mg/kg) (fig. 5).

Anti-inflammatory Study

Formalin-Induced acute inflammation

The sub-plantar injection of formalin, caused significant inflammation in the hind paws of the rats. The graded doses of 50, 100 and 200mg/kg of EtFSs were used for the formalin test. The 200mg/kg of EtFSs had a higher activity when compared with piroxicam (fig. 6).

Antipyretic study

Brewer's yeast Induced pyrexia in rats

The antipyretic effect of EtFSs in the model used in rats demonstrated significant and dose-dependent responses at $p<0.05$. The activity of EtFSs at all the graded doses was higher than paracetamol (150 mg) (table 8).

DISCUSSION

The percentage yields of the fractions were 1.04 % for HFSs, 6.64 % for EtFSs and 12.83 % for BFSs. The yield of the fractions increased with increasing polarity of the solvents. This trend is expected because, the more polar a solvent is, the more it extracts the phytochemicals (Halilu *et al*., 2013a; Ammar *et al*. 2017; Zhang *et al*, 2018: Halilu *et al*., 2020; Enev *et al*., 2021). The qualitative phytochemical screening of CrESs revealed the presence of tannins, flavonoids, triterpenoids/steroids and saponins. This observation agrees with Zehravi *et al*. (2022) who reported the presence of these compounds in other Scutellaria species with the exception of saponins. The presence of the saponins in *Scutellaria sibthorpii* may be due to its geographical distribution, soil chemistry and other environmental factors (Burneo *et al*., 2021). Furthermore, the qualitative phytochemical screening of EtFSs revealed the presence of the same set of compounds with the exception of tannin which was found to be absent in EtFSs. This observation may be due to the high polarity of tannins that might not have been portioned into the ethyl acetate. The high polarity of the tannins may be due to the poly hydroxyl groups on the aglycone and the hydroxyl groups on the sugar moieties since hydrolysable tannins are glycosides (Halilu *et al*., 2020).

The total phenolic contents (TPC) of CrESs and EtFSs were 5.7351mg and 5.1587mg gallic acid equivalents/mg of extract respectively. The result from this study differed from Gizem *et al*. (2022) who reported the phenolic content of 0.0295 mg GAE/mg extract of the infusion from *S. sibthorpii* which is lower when compared with the crude extract used in this study. Phenolic compounds have been reported to demonstrate antioxidant and anticancer activities (Bushra *et al*., 2009; Halilu *et al*., 2013b; Halilu *et al*., 2017). Dimitrios *et al*. (2023) stated that the antioxidant activity increases with an increase in the polarity of the extracting solvents (Sushant *et al*., 2019).

The total flavonoid content (TFC) of the CrESs and EtFSs were 0.4416 and 0.3734mg equivalent of quercetin/mg of extract. Flavonoids have been reported as anti-tumour agents acting as a free radical quencher. In addition to antioxidant activity, the inhibition of cancer development by phenolic compounds relies on a number of basic cellular mechanisms (Ammar *et al*., 2017; Abdelfatah *et al*., 2023).

Antioxidant activity of CrESs and the fractions demonstrated a great of their potentials in fighting diseases associated to free radicals. The qualitative and quantitative and quantitative assays demonstrated this fact. The qualitative screening of antioxidant activity on TLC plate using the DPPH free radical scavenging assay revealed preliminary evidence of antioxidant activity by the CrFSs and fractions (Plate 1 (A, B, C and D). The development of yellow spot against purple background of DPPH demonstrates antioxidant activity (Halilu *et al*., 2017). The HFSs demonstrated little discoloration indicating little activity which may be associated to the presence of little amount of free radical scavenging compounds. The DPPH free radical assay as a rapid, easy to handle and independent of sample polarity, it is the most convenient and quick method for rapid screening of plant extracts for radical scavenging activity (Koleva *et al*., 2002). The quantitative estimation of the DPPH free radical scavenging activities of CrESs and the fractions is shown in fig. 1. The CrESs and fractions demonstrated free radical scavenging activity with the highest potency observed from HFSs with IC_{50} (2.944mg/mL), then followed by BFSs (IC_{50}= 5.103mg/mL), EtFSs (IC_{50}=6.57 mg/mL) and the CrESs (IC_{50}=17.26mg/mL). Although the

result of this study differed from those of Gizem *et al* (2022) who reported the the high antioxidant activity of *S. sibthorpii* with IC_{50} value 0.000133mg/mL. The activity of the HFSs was same as that of the ascorbic acid (2.944 mg/mL) and this observation may be due to caratenoids (antioxidant) which occur in plant leaves along with chlorophyll which are known to be fat soluble and are readily extractable by n-hexane. Carotenoids are efficient natural antioxidants that can scavenge singlet molecular oxygen and peroxyl radicals. In the humans, carotenoids play a vital in the protection of the body against free radicals. DPPH is a stable free radical commonly used for investigating radical scavenging activity of phytochemicals. If the stable DPPH free radical accepts an electron from an electron donor, the violet color of the DPPH radical reduces to yellow colored diphenyl picrylhydrazine radical. Phytochemicals which are able to produce this reaction maybe regarded as antioxidants and therefore serve as free radical scavengers (Sasikumar and Pavithra, 2015). Therefore, the CrESs and the fractions demonstrated free scavenging activity in the order of HFSs, BFSs, EtFSs and CrESs with the least. The least activity demonstrated by the crude extract may be attributed to the inhibitory activities of various plant constituents when in combination.

In the H_2O_2assay, the CrESs and fractions showed free radical scavenging activities. The highest free radical scavenging activity with IC_{50} value of 0.4352mg/mL was observed in EtFSs followed by HFSs (1.026mg/mL), CrESs (1.234 mg/mL) and BFSs (26.81mg/mL). The IC_{50} value of CrESs and the fractions were compared with standard ascorbic acid ascorbic acid which had an IC_{50} value of 9.29mg/mL. The hydrogen peroxide scavenging effect revealed the highest effect from EtFSs with IC_{50} 0.4352 mg/mL, then followed by HFSs 1.026mg/mL and CrESs 1.234mg/mL. The ascorbic acid had IC_{50} of 9.29 mg/mL. $H_2O_2$is produced as a strong oxidant which can activate the cellular signaling pathway to stimulate cellular proliferation or differentiation. H_2O_2 is generated in a biological system by many oxidizing enzymes such as super dismutase (Sushant *et al*., 2019). The formation of H_2O_2 in high amount is responsible for oxidative stress and inflammation reactions, which are associated with many diseases such as cancer, diabetes and cardiovascular disorders (Pham-Huy *et al*., 2008; Mahmoud *et al*., 2011). This is a result of the rapid decomposition of H_2O_2 and subsequent generation of hydroxyl radical (*OH) which trigger lipid peroxidation and damage cell components (Saed-Moucheshi *et al*., 2014). H_2O_2 is not very reactive, but it can sometimes be toxic to cells because it can give rise to hydroxyl radicals. Thus, the removal of H_2O_2 as well as $O_2^{\cdot-}$ is very important for antioxidant defense in cell or food systems (Gulcin *et al*., 2004). Therefore, the scavenging activity of the CrESs and fractions demonstrated against of H_2O_2 may be attributed to the phenolic compounds, which donate electrons to H_2O_2, thus neutralizing it to H_2O (AsokKumar *et al*., 2009).

The β-carotene discoloration assay is dependent on the disappearance of the intense yellow colour of β-carotene due to its reaction with the radicals which are formed by linoleic acid oxidation in the emulsion. The rate of β-carotene discoloration can be reduced in the presence of antioxidants. The antioxidant activity demonstrated by the EtFSs was the highest with IC_{50} of 0.00014 mg/mL, followed by the CrESs was 1.124 mg/mL, then HFSs at 1.494 mg/mL and BFSs at 29.41 mg/mL. The antioxidant activity demonstrated by the EtFSs was far more than the ascorbic acid 2.85 mg/mL. This may be attributed to the flavonoid content of the ethyl acetate fraction (Vadivukkarasi and Pavithra, 2014; Lu *et al*., 2014). From the three models, it can be deduced that the antioxidant activity demonstrated by the extract/fractions could be due to the phenolic compounds. This is because, the phenolic hydroxyl groups confer free radical scavenging capacity on the compounds (Zhu *et al*., 1997; Halilu *et al*., 2013b; Nasution *et al*., 2022). According to Huma *et al*. (2022), the antioxidant activities of plant extracts are higher when in combination than when the constituents are separated.

The antibacterial activity demonstrated by CrESs and fractions at 20 mg/ml produced a zone diameter of 6.3 to 9.7 mm against the test bacteria. *S. aureus* was the most susceptible as the CrESs, EtFSs and BFSs inhibited its growth with zones of 9.7, 9 and 7 respectively. The susceptibility of the Gram-positive and Gram-negative bacteria to the CrESs/fractions differs due to structural differences in their cell envelope compositions (Halilu *et al*., 2008b). The outer cell membrane of the Gram-negative blocks the penetration of large molecules and hence their resistance to the CrESs/fractions. The crude extract inhibited all the organisms tested. These findings agree with results obtained when a crude extract of *Scutellaria orientalis* L. was tested on gram-negative bacteria and gram-positive bacteria (Yılmaz *et al*., 2020). Furthermore, the EtFSs was the most active against the bacteria. The EtFSs was the most active on *S. aureus* (9 mm). The result of this study agrees with earlier findings by Dereboylu *et al*. (2012) on *S. sibthorpii* from Cyprus who reported from their study that the ethyl acetate extract was most effective on *S. aureus* with MIC of about 10 mg/mL. But, the MIC of the ethyl acetate fraction from the current study on *S. aureus* was 1.25 mg/mL. Interestingly, the EtFSs from the current study produced the same MIC (1.25 mg/mL) on *S. aureus* (Gizem *et al*., 2022). The activity of ethyl acetate extract from other species of Scutellaria against *P. aeruginosa* and *B. cereus* have been documented (Gizem *et al*., 2022). The activity may be due to saponins, flavonoids and tannins (Segaran *et al*., 2023). Silva *et al*. (2018) reported that plant-derived compounds exert their antibiotic potential through

synergism. Furthermore, according to Martin *et al*. (2023), the multi-component mixture of plant extracts enhances antibacterial activity due to a synergistic effect (Shahabe *et al*., 2021; Huma *et al*., 2022). This suggests that sometimes when plant compounds are separated from each other, there may be a decrease or loss of activity. Bioactive compounds found in plant extracts are complex mixtures, their isolation from one another creates problems such as decrease or loss of activity (Sasidharan *et al*., 2011). The result of this study suggests that the ethyl acetate fraction contains compounds with antibacterial activity that can be investigated for the development of novel antibiotics.

The safety evaluation of any plant used for therapeutic purpose is meant to establish the nature and significance of the adverse effects (Ibrahim *et al*., 2016). The LD_{50} is used to determine the safety or toxicity of a substance (Renata and Patrick, 2022). The results of the acute toxicity study indicated that the CrEss/fractions of *Scutellaria sibthorpii* leaf administered through the oral route to mice in phases 1 and 2 using Lorke's method did not produce any mortality in the mice. This observation indicated that the extract and fraction are relatively safe (Chinenye *et al*., 2019; Halilu *et al*., 2020). Therefore, the LD_{50} was greater than 5000mg/kg and thus classified as relatively safe (Renata and Patrick, 2022; Halilu *et al*., 2023).

The peripheral nociception effects of CrESs, EtFSs and BFSs on acetic acid induced writhing in mice were ascertained. The ethanoic acid induces an increase in peritoneal fluids of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ as a result of abdominal contractions, dorsoabdominal muscle twisting and sensitization of peripheral chemosensitive nociceptors (Dirig *et al*., 1998), which leads to the development of pain (Bley *et al*., 1998). The CrESs/fractions in the current study exhibited significant ($P<0.05$) analgesia in the ethanoic acid-induced abdominal constrictions in mice, indicating that they could possess both central and peripherally mediated analgesic activity. The trend indicated in the analgesic activity of the CrESs and fractions was dose-dependent. The EtFSs demonstrated strong analgesic effect in the same order of magnitude as that observed after piroxicam administration at 10 mg/kg. The mechanism of action is thought to involve, in part, local peritoneal receptors (Musa *et al*., 2009). The CrESs and fractions may have interfered with these peritoneal receptors to bring about analgesia (Musa *et al*., 2009).

The EtFSs exhibited greater protection than all the samples tested and to further confirm its analgesic activity, it was investigated for its centrally acting effect. Therefore, its analgesic activity was tested using the hotplate-induced pains (Alhadeff *et al*., 2018). A report has indicated that drugs act centrally such as piroxicam exhibit this action in both central and peripheral. This investigation has revealed that the EtFSs has the ability to block the receptors of pain on hotplate-induced pains at higher doses indicating that EtFSs has both central and peripheral analgesia similar to piroxicam. This activity expressed by EtFSs may be due to steroids, triterpenoids and other phenolic compounds.

The formalin-induced nociceptive response produces a biphasic pain response and is very useful in the assessment of pain-relieving efficacy and mechanism of analgesic action of the test drug (Jimoh *et al.*, 2011, Kumar and Jain, 2014). There are two distinct phases which constitute the formalin test. The first phase of the neurogenic pain usually peaks at 5 minutes after injection of formalin due to chemical stimulation of nociceptors of sensory afferent C-fibres. The second phase normally peaked at 15-30 minutes after the injection of formalin and these represent the neurogenic and inflammatory pain responses, respectively and are mediated by a combination of NMDA (*N-methyl-D-aspartate receptor)* and non-NMDA receptors in the peripheral input and spinal cord sensitization (Hunskaar and Hole, 1987). Centrally-acting drugs such as narcotics inhibit both phases of formalin-induced pain while peripherally-acting drugs inhibit only the second phase (Ugwah-Oguejiofor *et al.*, 2013; Yang *et al*., 2014). The formalin-induced pain test has been used to investigate the analgesic activity of the EtFSs. The result revealed that pretreated animals with EtFSs produced significant antinociceptive effects in the early phase compared to the control group. The EtFSs at 200 mg/kg produced great activity and piroxicam (10 mg/kg) produced significant activity as indicated by a reduction in the licking time in the second phase of the nociception (fig. 5). This suggests that the analgesic effect of EtFSs may possibly be mediated through the inhibition of prostaglandin synthesis and this may be due to steroids, triterpenoids and flavonoids. The Formalin-induced paw oedema in mice is a simple experimental model of sub-chronic inflammation used to screen anti-inflammatory agents. The EtFSs produced a better anti-inflammatory activity than piroxicam in reductions of oedema development.

Drugs used in treatment of fever are known to elicit their actions by antagonizing cyclooxygenase activity by rise in prostaglandin E_2 (PGE_2) and in return masks high temperatures. Sick individuals suffering from infections and damaged experience rise in body temperature (Eugene *et al*., 2022). The mediators in this process such as interleukins can be activated which could lead to elevated body temperature due formation of prostaglandin E_2 (Eugene *et al*., 2022). This study has shown that EtFSs at 200 mg/Kg most significantly decreased the rectal temperature of rats similar to paracetamol. This suggests that the EtFSs could control fever as it stops inflammatory symptoms centrally and peripherally. It may break down

pyrogenic releasing cytokines as they also decrease the formation of PGE_2 from cyclo-oxygenase possibly via the same mechanism as paracetamol. The observed antipyretic activity may be due to steroids, triterpenoids and flavonoids (Bantayehu and Agumas, 2023).

CONCLUSION

Scutellaria sibthorpii crude extract (CrESs) and fraction demonstrated antioxidant, antibacterial, analgesic, anti-inflammatory and antipyretic activities. The activities have been attributed to the phytochemicals therein. The EtFSs performed better than all the fractions in the biological activities investigated. The EtFSs exhibited higher antioxidant activity with IC_{50} values greater than the ascorbic acid in all the experimental models. The EtFSs had better antibacterial activity on *S. aureus*. The LD_{50} of the crude extract/fractions were greater than 5000 mg/kg and thus classified as relatively safe. The EtFSs showed significant dose-dependent analgesia in the ethanoic acid-induced writhing in mice. The EtFSs exhibited central and peripheral analgesia similar to piroxicam. The study revealed that, the EtFSs at 200 mg/Kg significantly reduced the rectal temperature of rats similar to paracetamol. The study has justified the rationale for the traditional use of *Scutellaria sibthorpii* in management of bacterial infections, pains, inflammation and fever.

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