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Evaluation of Nutraceuticals and Antimicrobial Activities of *Achomanes difformis* Extract – Honey Mixture as Potential Antitussive Agent

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Abstract*: Antitussives are substances with potentials of suppressing or treating cough and associated respiratory tracts disorders. Conventional cough drugs pose controversies of habit forming potentials amongst other side effects. Achomanes difformis, a natural plant was assessed for their potentials as antitussive agent. Achomanes difformis extract – honey mixture was prepared and nutraceuticals were determined using standard analytical methods with Tutolin cough syrup employed as standard. Antimicrobial activity of test samples against selected bacteria were investigated. Agar well diffusion method was employed for inhibitory activity of Tutolin Syrup and Achomanes difformis extract - honey mixture on isolates. Mineral composition of Tutolin and Achomanes difformis extract – honey mixture in mg/100g showed Na (12.01 ± 0.02, 18.20 ± 0.01), K (56.00 ± 0.01, 96.00 ± 0.02), Ca (10.00 ± 0.02, 6.00 ± 0.02), Mg (5.64 ± 0.04, 14.40 ± 0.02), Zn (0.02 ± 0.01, 0.05 ± 0.01), P (1.00 ± 0.01, 2.00 ± 0.01) respectively, Ni and Pb were not detected. Phytochemicals of Tutolin cough syrup and extract - honey mixture showed phenol (%) (1.36 ± 0.02, 2.68 ± 0.03), glycosides in mg/100g (0.01 ± 0.01, 5.38 ± 0.05), saponins (%) (0.84 ± 0.01, 1.12 ± 0.02), flavanoids (%) (0.01 ± 0.01, 0.01 ± 0.01). Steroids, alkaloids, anthraquinones and terpenoids were not detected. The results showed that extract - honey mixture inhibited bacteria growth isolates and compared favourably with the reference antibiotics indicating the potentials of A. difformis extract-honey mixture in inhibiting the bacteria causing lung infections, hence, can be exploited industrially as antitussive agent*

Keywords—*Achomanes difformis*, Antimicrobial, Antitussive, Nutraceuticals, Phytochemicals, Tutolin

1. INTRODUCTION

Since time immemorial, man has relied upon the usage of medicinal plants for the maintenance of health. The traditional use of medicinal plants for curing and prevention of illness, promotion of both physical and spiritual well-being among human beings in the South – Western Nigeria have become paramount in almost every house – hold. The importance of medicinal plants has grown exponentially now that the world is turning to plants which are sources of drugs supplements and herbal
preparation for the management of human health [1] (Mahabir and Gulliford, 1997).

Achomanes difformis, belongs to the family *Araceae*, an herbaceous plant with prickly stem having huge divided leaf and spatial that rises from a horizontal tuber occurring in the forest of West African (Tapas *et al.*, 2008) [2] and it is called 'Ogirisako' in the South – Western Nigeria (Soladoye *et al.*, 2005)[3]. The rhizome can be eaten only after a special preparation that requires a prolonged washing and cooking (Aliyu *et al.*, 2008)[4].

Honey is regarded as the nectar and saccharine which exudates form the plants which is gathered and stored in the honey comb by honey bees (Peter *et al.*, 2007)[5]. Honey is known to improve food assimilation and has been found useful in chronic and intestinal disorders such as constipation, duodenal ulcers (Peter *et al.*, 2007)[5].
Antitusissives are drugs that has the potentials of suppressing cough.

The present study aims to prepare *Achomanes difformis* extract – Honey mixture in the ratio 1:1 by dispensing 5ml of the extract of *Achomanes difformis* into the 5ml of Honey with proper agitation on a mechanical shaker. The *Achomanes difformis* extract – Honey mixture prepared will thereafter be evaluated for minerals, phytochemical, antinutrients compositions and antibacterial activities of the prepared mixture against selected bacterial isolates and compare the results with the standard Tutolin cough syrup to ascertain its antitussive properties.

2. MATERIALS AND METHODS

2.1 Preparation of *Achomanes difformis* extract and *Achomanes difformis* extract – Honey mixture

A. difformis sucker was washed with clean water. It was thereafter cat into pieces with a sterile knife and put into a juice extractor to extract the juice. The juice was sieved and the clean milky juice obtained was used for the misture.
Achomanes difformis extract – Honey mixture in the ratio 1:1 was prepared by dispensing 5ml of the extract of *Achomanes difformis* into the 5ml of Honey and thereafter put in a homogenizer to obtain a single phase.

2.2 Minerals Analysis

The minerals were analyzed by ashing the samples; ADH and TCS at 55^0C to constant weight and dissolving the ash in volumetric flask using distilled – deionized water with a few drops of concentrated hydrochloric acid. Sodium and Potassium were determined using a flame photometer (Model

405 UK) using NaCl and KCl for the standards preparation. All other metals such as Calcium, Magnesium, Zinc, Nickel, Lead and Phosphorous were determined by Atomic Absorption Spectrophotometer (Perkin – Elmer Model 403, Norwalk CT, USA).

2.3 Antinutrient Analysis

Phytic acid and phytate were determined according to the method described by Okwu (2005)[6]. Oxalate content was estimated by titrimetric method as modified by Akindahunsi and Salawu (2005)[7]. The tannins content was determined using the methods of Makkar *et al. (*1993)[8]. All determinations were done in triplicates.

2.4 Phytochemical Screening

Phytochemical constituents; phytate, phytic acid, oxalate, tannins, saponins, flavonoid, glycosides, alkaloids, steroids, anthraquinones and terpenoids of samples ADH and TCS were screened and determined quantitatively as described by Sofowora (2006)[9].

2.5 Test Organisms

The followings clinical bacteria isolates: *Pseudomonas syringine, Staphylococcus aureus, Xanthomonas campestris, Enterobacter aerogenes, Bacillus cereus, Bacillus subtilis, Klebsiella pneumoniae, Mycobacterium tuberculosis, Salmonella typhi* and *Escherichia coli* used were sourced from the Medical Laboratory Unit of Obafemi Awolowo Teaching University Hospital, Ile – Ife from infected wounds. All the organisms were identified through their colonial characteristics, gram staining and biochemical tests following the scheme outlined in Bergey's manual (Bergy and Holt, 1993)[10].

2.6 Preparation of Culture Media

The nutrient agar media was used for the antibacterial activities. The media was prepared according to manufacturer's direction. The media was sterilized by autoclave at 121^0C for 15 minutes before usage.

2.7 Antibacterial Susceptibility Test

The antibacterial activities of the samples ADH and TCS on the test bacterial isolates were determined by employing the agar diffusion method as described by Irobi *et al.,* 1994 [11] as modified by Murray *et al.,* 2004[12]. Twenty milliliters of sterile nutrient agar in petri dishes were seeded with 20mg/L of samples ADH and TCS using sterile cotton swabs. Wells of 3.0mm in diameter were cut out on the seeded plates using sterile cork borer. The samples were allowed to diffuse into the medium and the plates were incubated at 37^0C for 24hours. The zones of inhibition which indicated the effects of the antibacterial activities were determined around the wells according to the method of Murray *et al.,* 2004[12] and standard reference antibiotic drugs were used as control.

Table 1. Mineral composition of *Achomanes difformis* extract – honey mixture and tutolin cough syrup

Minerals	**Concentration (mg/100g)**	
	ADH	**TCS**
K	$96.00^a \pm 0.02$	$56.00^a \pm 0.01$
Na	$18.20^a \pm 0.01$	$12.01^a \pm 0.02$
Ca	$6.00^a \pm 0.02$	$10.00^a \pm 0.02$
Mg	$14.40^a \pm 0.02$	$5.64^a \pm 0.04$
Zn	$0.05^a \pm 0.01$	$0.02^a \pm 0.01$
Ni	ND	ND
Pb	ND	ND
P	$1.00^a \pm 0.01$	$1.00^a \pm 0.01$

ADH: *Achomanes dofformis* extract-Honey mixture
TCS: Tutolin Cough Syrup
a: Means of triplicate determination
ND: Not Detected

Table 1 showed the minerals composition of *Achomanes difformis* extract-Honey mixture and Tutolin cough syrup in mg/100g. The results of the analysis showed a high concentration of Potassium (K) in both samples. Zinc (Zn) and Phosphorus (P) were present in low concentration in both samples. The presence of Phosphorus in the samples showed the role of Phosphorus for growth and repair of body cells and tissues. Also, the presence of Magnesium (Mg) in the samples indicated the potential of the *Achomanes difformis* extract-Honey mixture in the prevention of cardiovascular disease (Del *et al.,* 2015)[13]. The absence of Nickel (Ni) and Lead (Pb) in both samples is an indication that the consumption of the mixture do not pose any treat to the consumers.

Table 2. Antinutrient components of *achomanes difformis* extract–honey mixture and tutolin cough syrup in mg/100g

Parameter	**Composition, mg/100g**	
	ADH	**TCS**
Phytate	$3.75^a \pm 0.02$	$2.26^a \pm 0.06$

Phytic Acid	1.05 [a] ± 0.03	0.64[a] ± 0.08
Oxalate	16.48 [a] ± 0.03	9.90[a] ± 0.04
Tannins	1.07 [a] ± 0.02	0.52[a] ± 0.03

ADH: *Achomanes difformis* extract-Honey mixture
TCS: Tutolin Cough Syrup
a: Means of triplicate determination

Table 2 depict the antinutrient components of *Achomans difformis* extract-Honey mixture and Tutolin cough syrup. The Phytates, Phytic Acid, Oxalate and Tannins content in the *Achomanes difformis* extract – Honey mixture were higher than that of the Tutolin cough syrup. The phytic acid chelates with di and trivalent metal ions such as Cu, Mg, Zn and Fe to form poorly soluble compound that are not readily absorbed in the gastrointestinal tract. Thus, phytic acid interferes with the bioavailability of minerals. Hence, the low value of phytic acid observed in both samples showed that the metals will be available for body metabolism (Aletor *et al.*, 2002)[14].

Generally, the closeness of the antinutrients content in both samples showed the potential of the *Achomanes difformis* extract – Honey mixture in the treatment of cough infections just like the Tutolin cough syrup.

Table 3. Phytochemical screening of *achomanes difformis* extract–honey mixture and tutolin cough syrup

Phytochemicals	**ADH**	**TCS**
Saponins	+++	+++
Tannins	-	-
Flavonoid	+	+
Glycosides	+++	+++
Alkaloids	-	-
Anthraquinones	-	-
Steroids	-	-
Terpenoids	-	-

ADH: *Achomanes dofformis* extract-Honey mixture
TCS: Tutolin Cough Syrup
+++: Present in Appreciable Amount
+: Present in Minute Amount
- : Absent

Table 3 depict the phytochemical screening of *Achomanes difformis* extract – Honey mixture and Tutolin cough syrup. The results of the analysis showed the presence of Saponins, Flavonoid and glycosides while Tannins, Alkaloids, Anthraquinones, Steroids and Terpenoids were not determined or found absent in both samples. The flavonoids occur as glycosides, glycones and methylated derivatives. The major active nutraceutical ingredients in plants are flavonoids (Tapas *et al.*, 2008)[2] and flavonoids are potent water soluble antioxidants and free radical scavengers that prevents oxidative cell damage, having strong anti-inflammatory and anticarcinogenic activities (Okwu, 2004)[15].

Table 4. Quantitative phytochemicals of *achomanes difformis* extract–honey mixture and tutolin cough syrup in mg/100g.

Phytochemicals	**ADH (mg/100g)**	**TCS (mg/100g)**
Phenol	2.68 ± 0.02	1.30 ± 0.02
Glycosides	5.38 ± 0.05	3.01 ± 0.01
Saponins	2.84 ± 0.02	2.64 ± 0.01
Flavonoids	0.01 ± 0.01	0.01 ± 0.01

ADH: *Achomanes dofformis* extract-Honey mixture
TCS: Tutolin Cough Syrup
ND: Not Detected

Table 4 depicts the quantitative phytochemicals of *Achomanes difformis* extract – Honey mixture and Tutolin cough syrup in mg/100g. The result of the analysis showed the presence of Glycosides, Saponins in both samples. Flavonoids occur as aglycones, glycosides and methylated derivatives. As is typical for phenolic compounds, they can act as potent antioxidants and metal chelators. They also have

a long been recognized to possess anti-inflammatory, antiallergic, hepato-protective, anti-thrombotic, antiviral and anticarcinogenic activities (Harborne, 1988)[16].

Table 5. Antibacterial activities of *achomanes differmis* extract – honey mixtures and tutolin cough syrup against some selected bacteria isolates.

	Zones of inhibition (mm)						
Test microorganism	**ADH**	**TCS**	**A**	**B**	**C**	**D**	**E**
Staphylococcus aureus	10.50	14.00	20.00	14.00	15.00	16.00	19.50
Xanthomonas campestris	16.00	19.20	28.00	14.00	24.00	16.00	30.00
Pseudomonas syringine	16.00	16.40	19.75	15.00	24.00	16.00	21.00
Bacillus subtilis	11.50	13.00	21.00	16.50	24.00	16.00	20.00
Klebsiella pneumonia	7.75	10.00	22.00	20.00	24.00	16.00	20.00
Enterobacter aerogenes	8.00	7.00	20.00	14.00	14.00	8.00	14.00
Bacillus cereus	16.00	17.50	20.00	14.50	13.50	13.00	14.00
Salmonella typhi	15.50	14.00	21.00	13.50	13.50	8.00	13.50
Escherichia coli	15.00	14.00	20.00	16.50	15.00	16.00	18.50
Mycobacterium tuberculosis	16.20	14.50	16.50	14.20	15.50	14.00	18.00

ADH: *Achomanes dofformis* extract-Honey mixture
TCS: Tutolin Cough Syrup
A: Amoxicillin
B: Gentamycin
C: Septrin
D: Streptomycin
E: Ampiclox

Table 5 depicts the antibacterial activities of *Achomanes differmis* extract – Honey mixture and Tutolin cough syrup against some selected bacteria isolated. The results of the analysis showed that all the bacteria were sensitive to the *Achomanes differmis* extract – Honey mixture and similarly with the Tutolin cough syrup. The samples of graded concentration of 10mg/L exhibited graded effects on the microorganisms and similarly all the standard reference antibiotic concentration of 10mg/L of Amoxicillin, Gentamycin, Septrin, Streptomycin and Ampiclox used as control drugs inhibited the growth of all the selected bacteria under study at various zones of inhibitions.

3. CONCLUSION

The *Achomanes differmis* extract – Honey mixture contain phytoconstituents of medicinal importance and of low antinutritional status with nutritionally valuable minerals as well as good antibacterial activity against the bacterial isolates such as *Mycobacterium tuberculosis, Escherichia coli*. The results compared favourably with the standard cough syrup (Tutolin). Hence, *Achomanes differmis* extract – Honey mixture can be exploited industrially for human benefits as antitussive agent.

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