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Editorial

The chief editor on the behalf of the editorial board has great pleasure in presenting July 2020 issue of European International Journal of Science and Technology (EIJST) to the research community and the world at large. EIJST aims to create a platform between the people who seek to publish their work and the people who wise to keep up with latest finding in the areas of science and technology. The journal provides opportunities to the researchers, academicians, and professional to publish their research paper around the world.

The quick review process, rich editorial board and quality publications might make this journal unique. The journal focuses on double- blind review process. It is published in both print and online forms.

Although numerous research are being made by the scholars, academicians and professionals, especially in developing countries, there are a lot of problem towards the publication of research finding due to high excessive author's fee, lengthy review process and complex terms and conditions of the publisher. EIJST provides unique opportunities to the researcher, academician and professional in this regard.

The chief editor is very grateful to the members of the editorial board for their kind response toward the establishment of such type of attempt.

We seek the blessings and support of all towards the journey of the journal.



Dr. Laurent Mathy

Chief Editor,

European International Journal of Science and Technology

Table of Content

Title	Page
Use of Clove Carrot Mix in Cookies; A Way Towards Development of Low Caloric and High Fiber Cookies with Improved Organoleptic Properties <i>Noor-e-Huma¹, Dr. Nadia Akram² and Dr. Rabia Naz³</i>	1-15
Synthesis, Characterization, and <i>in Vitro</i> Antimicrobial Screening of Some Novel Heterocycles Linked to Tetrazolo[5,1-f]-1,2,4-Triazine Moiety <i>Mamdouh A. M. Taha</i>	16-25
Use of Rice Husks as partial replacement of coarse aggregates in concrete paving blocks <i>Mwalimu K. Musau¹, Douglas Shitanda², Michael Githinji³, Caroline Mwendu⁴</i>	26-34

Use of Clove Carrot Mix in Cookies; A Way Towards Development of Low Caloric and High Fiber Cookies with Improved Organoleptic Properties

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Abstract

Cookies are an easy to go snacks with an increasing demand owing to their ease of consumption in this era of changed dietary patterns and sedentary life style. The effect of clove substitution in carrot flour cookie was assessed. The carrot flour being a good source of high fiber and low-calorie ingredient was used to assess the organoleptic properties of the cookies. The composite flour consists of carrot flour and white flour in two different ratios as; 50:50% (CF1) and 70:30 % (CF2), respectively with 2.5g of clove added to each. The best selected ratio after sensory evaluation was first analyzed for its nutritional composition, standardized for a replicable model and tested for the proximate estimation. Proximate analysis revealed a considerable change in crude protein (6.60% to 9.46%), crude fiber (4.10% to 5.80%). Clove incorporation improved the sensory quality of composite flour cookies and can open new opportunities for development of functional products with added health benefits specifically the snack items like cookies.

Keywords: Clove, nutritional composition, proximate analysis, therapeutic value, aromatic plant, beta-carotene, carrot flour.

1. Introduction

In the present world, nutraceuticals have gained great attention owing to their nutritional properties, safety as well as therapeutic effects. The term “Nutraceutical” refers to a substance that is derived from a food source that consist of dietary benefit of a disease and the already present base nutritional values present in that food. (Zeisel, 2018). ¹Development of snack item with less energy density are on high rise and carrot pomace can be good source of it in this regard as it may not only help in waste utilization but also improve nutritional quality of cookies. Besides, spices are rich source of antioxidants and amongst them, clove is one. Its usage is limited to the ethnic foods only, but can be added in snack items such as cookies, so need to look for ways by which clove can become part of such type of snacks. About 15-20% essential oil is present in a good quality clove bud with three most prominent volatile essential oils. The plant possesses one of the richest sources of phenolic compounds such as eugenol, eugenol acetate and gallic acid. (German, 2017).

Cloves are commercially harvested in India, Indonesia, Pakistan, Tanzania and Sri Lanka. It is used for medicinal purpose like different therapeutic and dental complaints in China and Western countries (E W Riptanti, 2018). The plant is also used to treat various gastrointestinal disorders such as diarrhea, vomiting, nausea, cough, dyspepsia, flatulence, stomach distention, gastrointestinal spasms along with uterine contractions and stimulate nerves. (Mahmoud S. M. Mohamed, 2018). The essential oils derived from the plant not only contribute to aromas and flavor enhancers but also prevent several diseases caused by free radicals such as cancer.

Clove oil has been termed as “Generally Regarded as Safe” substance by the United States Food and Drug Administration. (Jian-Guo Xu, 2016). Eugenol and eugenol acetate are part of clove and act as antioxidants. Eugenol dose dependently binds to membranes thus, stabilizing them and protecting them against free radicals and invading agents. Compound **3** inhibits lipid oxidation and helps to limit structural changes to various tissues, such as the heart, kidney, and liver.

¹ According to Food and Drug Administration, a product can bear the structure of a functional claim if the claim is derived from the nutritive value itself. However, if the claim describes the structure of the function that is not related to the nutritive value of the product, the claim can only be made if the product complies to the marketing requirements as a dietary supplement or drug. (Ross, 2000)

Compound **3** inhibits histamine release from mast cells to reduce hypersensitivity besides having anti-anaphylactic and antispasmodic properties. Cloves inhibit oxidative tissue damage and cataract formation in the eye lens of rats (Daniel Pereira Bezerra, 2017).

Three kinds of volatile liquid fats are yielded from clove, oil extracted from the leaves, the stem and the buds. These are different from each other in terms of yield and quality. 1-Phenyethyl acetate is present in the clove bud essential oil and also in the grounded clove buds. (Klaus Gassenmeier, 2017). It is commonly identified as a flavoring agent.

About 15-20% essential oil is present in a good quality clove bud. The oil is ruled by β -caryophyllene (11.54%), eugenol acetate (1.76%), caryophyllene oxide (4.29%) and lastly eugenol that is present in highest amounts (76.23%), respectively. These makes up the 99% of oil. (Jian-Guo Xu, 2016). β -caryophyllene, that was the artefact of the distillation, has been reported to be a constituent of bud oil. Other essential constituents of clove oil are acetyl eugenol, vanillin crategolic acid tannins such as bicornin², methylsalicylate, gallototannic acid, flavonoidseugenin, rhamnetin, kaempferol, and eugenitin, triterpenoids such as oleanolic acid, campesterol and stigmasterol, and several sesquiterpenes. (Md. Azir Uddin, 2017). Eugenol (EUG, 4-allyl-1-hydroxy-2-methoxybenzene) a natural phenolic compound has been very well investigated for its pharmacological effects, and studies have shown it to possess significant antioxidant, anti-inflammatory, cancer-preventive, analgesic, and local anesthetic activity. (Liang-Liang Zhang, 2017)

Carrots (*Daucus carota*) is a traditional local grown vegetable in Pakistan. It is popular partly due to its freshness and due to the bulk, it provides in meals/salads. Carrots contain 50% β -carotene and could successfully be used as a supplement in cakes, cookies, dough and other types of functional products. The nutritional content of carrot is reported to be high especially amino acids, fatty acids and minerals. The presence of high amount of β -carotene in carrots makes it inhibit free radical scavenger, anti-mutagenic and immunity booster. Carrots are also a prominent source of calcium pectate and exceptional pectin fiber that has cholesterol reducing properties, anti-carcinogenic, anti-atherosclerotic and anti-hypertensive properties. (J. A. Ayo, 2017).

For the current study, clove was selected as the functional ingredient to be in cooperated in carrot flour cookies. Cookies were selected as the experiment product because of its popularity among the household consumption as a favorite tea time snack (Kehinde, 2017). Clove is one of the many nutraceuticals available to human kind.

² Bicornin is an ellagitannin found in the cloves and has a luteic acid group.

2. Material & Method

The current study was an experimental research which is designed to replace the all-purpose flour cookies with composite flour (CF) using carrot flour (*Daucus carota subsp. Sativus* belonging to *Apiaceae* family) with addition of clove (*Syzygium aromaticum*) as a bioactive compound. The study is quantitative and conducted in a sequential procedure, involving product development, product's sensory evaluation, product standardization, nutritional evaluation followed by proximate analysis. The product that was accepted by the sensory evaluators was tested for the proximate analysis as experimental product. The proximate was conducted for a clove-enriched, experimental "E" cookie and clove-excluded control "C" cookie, in Biotechnology Lab, using standard protocols and procedure of AOAC Methods. (AOAC Scientific Solutions, Standards & Methods, n.d.).

2.1 Product Development

Treatment plan for the composite flour (table 1 here)

2.1.1 Selection of Product

Carrot Flour was selected as the base ingredient for cookie. And, clove was added as a bioactive ingredient to the cookies. The experiment was done on cookies being the popular snack food by children and adults (Kehinde, 2017). Fresh carrots and cloves were purchased from the local market of Lahore city, Punjab, Pakistan.

2.1.2 Preparation of Ingredients

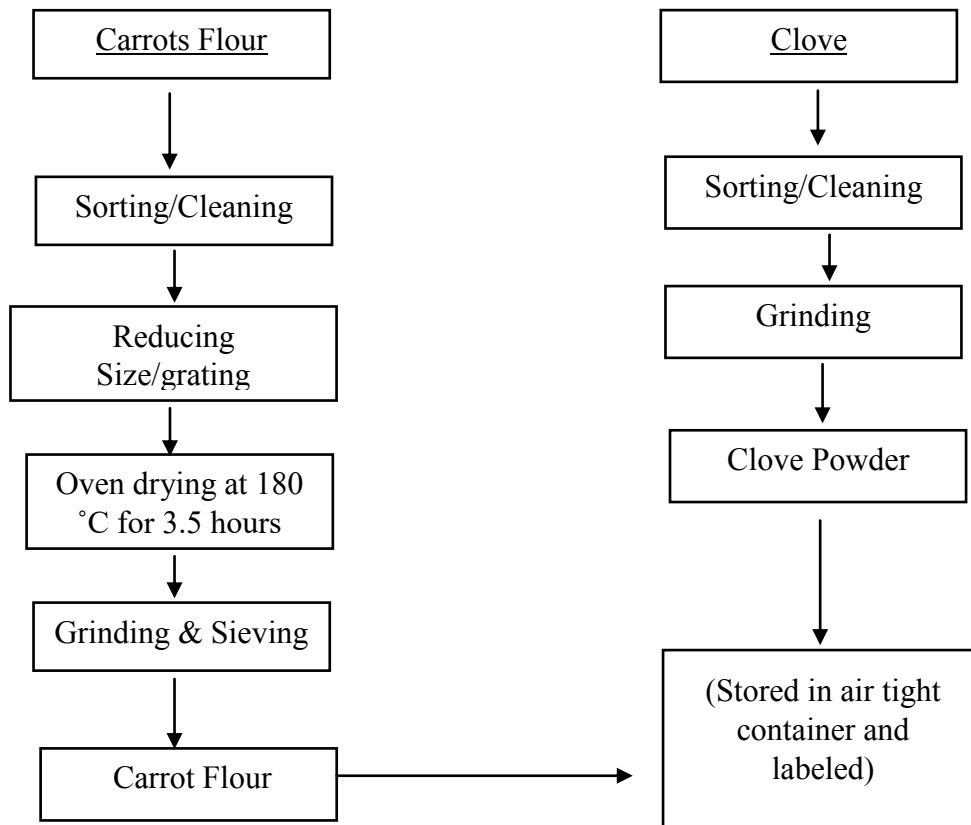


Figure 1: Flow Diagram for the Preparation of Ingredient

2.1.3 Development of Carrot Flour (figure 6 here)

2.1.4 Preparation of Cookies

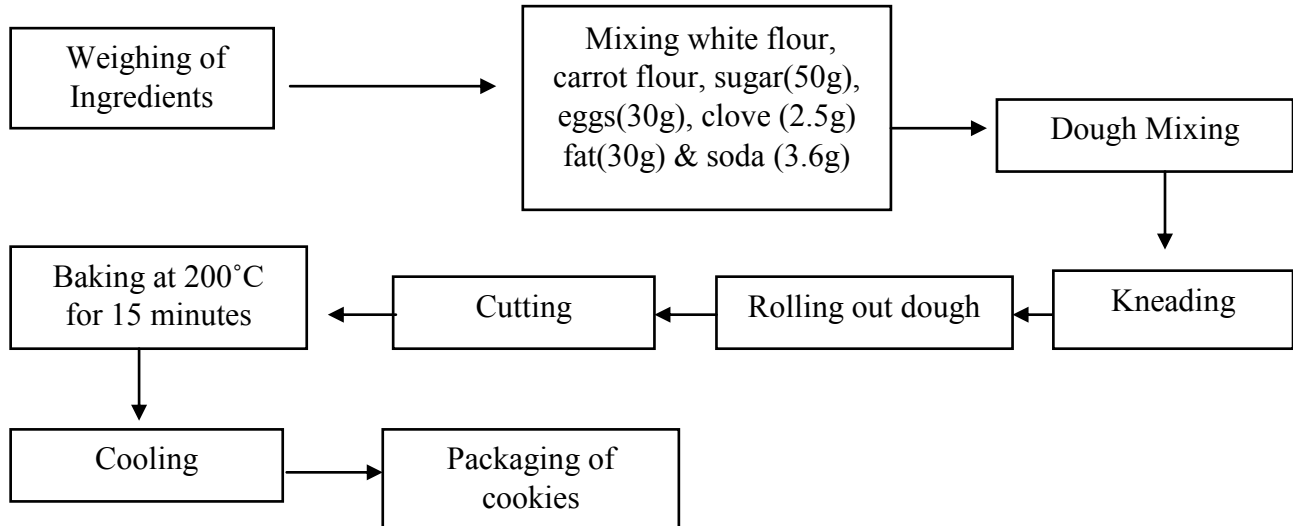


Figure 2: Flow Diagram for the Preparation of Cookies

2.2 Sensory Evaluation and Standardization

The sensory evaluation of the product was carried out by 8 trained panelists who were selected from the faculty of Food Science and Human Nutrition department Kinnaird College for Women – Lahore, Pakistan, based on their familiarity with the cookies. The cookies were appropriately coded as “A” and “B” of the same size. The panelist rinsed their mouth with fresh water after tasting of the first sample, before moving onto the next sample. They were not allowed to make comments during evaluation to avoid biasness. Sensory evaluation was based on a 9-point hedonic scale with 1-Extremely Dislike to 9-Extremely Like. The product food characteristics were flavor, aroma, color, texture and overall acceptability. The accepted product was standardized for a replicable model and all the changes provided by the panelist, were incorporated and a standard recipe for clove enriched carrot cookie was developed.

2.3 Nutritional Value

The nutritional contents of the most acceptable cookies were analyzed. The analyses included the determination of energy, protein, amino acids, carbohydrate, fat, vitamins and mineral contents. These analyses were performed according to the procedures described in Official Methods of Analyses of the Association of the Official Chemists. (AOAC Scientific Solutions, Standards & Methods, n.d.)

2.4 Proximate Analysis for cookies

Proximate analysis was conducted in the University of Veterinary Sciences Lahore (UVAS) Pakistan, for the control product (Carrot flour cookies without clove) and the experimental product (Carrot flour cookies with clove). The experimental product chosen for proximate was based on the most preferred between CF1 & CF2 product during the sensory evaluation. Proximate parameters

(carbohydrate, crude fats, crude protein, crude fiber and ash) of the clove were determined using the Association of Official Analytical Chemistry Method(AOAC Scientific Solutions, Standards & Methods, n.d.) in an automated machine in (UVAS). Calories were calculated for the control and experimental group using the formula. Crude protein was calculated using the Total Kjeldahl method. The NFE was calculated using the equation given below:

NFE= [100-% (moisture +crude protein +crude fat +ash +crude fiber)]
(AOAC Scientific Solutions, Standards & Methods, n.d.)

2.5 Data Analysis

For the compilation of data, Microsoft Excel (2013) was used.

3. Results and Discussion

The carrot cookies enriched with clove were developed, nutritionally evaluated, standardized, sensory assessed for their sensory parameters and tested for proximate analysis. “CF1” represents 50:50 ratio while “CF2” represents 70:30 ration, the former being the carrot flour and the latter being the all-purpose flour, respectively. Standardization was done thrice in accordance to panelist’s suggestions. Any suggestion made first time were incorporated in the next attempt to obtain the standardized recipe that can be replicated.

The sensory characteristics of cookies upon array of parameters were evaluated on the basis of flavor, aroma, color and texture and are shown in figure 3. The sensation of flavor perceived in the mouth and throat on contact with pieces of cookies was evaluated and the results are shown. CF2 cookies scored more in terms of sweetness (5:7) and richness (7:7). This is particularly due to the sweetness of the carrot flour present in greater quantity in CF2. The bitterness of CF2 was greater (5:7) owing to the presence of clove in the cookies. Although the clove quantity for both the cookies were same but the aroma was pronounced in terms of spicy-ness (4:6) and caramelly-ness (6:8) in CF2 owing to the presence of clove in cookies. The intensity or saturation of the color displayed not much diversion. Colors of the cookies were analyzed upon darkness, lightness and neutrality. CF2 were darker than CF1 scoring 4:6. This is particularly due to the presence of carrot flour in cookies which resulted in darker tone of CF2. The cloves were again present in equal quantity in both the cookies. The results of the texture appearance and mouth feel of the cookies was also analyzed. The addition of the clove to the cookies containing carrot flour did not much affect both the texture including the crunchiness and softness of the cookies, hence scoring 7:8 for both the parameters. While, crumbliness of the cookies scored 6:8.

Figure 4, shows the cookies with overall acceptability score. CF2 had greater degree of acceptance than CF1. Similar records were recorded in the results of the research by (Omachi .D.O, 2017) where cookies with more carrot flour and 2.5g of clove was more acceptable as compared to other treatments. It is evident in both the researches that if the composition of carrot flour increases, it is preferred by the panelists. It is also obvious from the results that supplementation significantly affected the overall acceptability of cookies. The maximum score recorded was 8.0 prepared from CF2 composite flour.

Cookies having higher sensory score were then standardized thrice to get the best response of all sensory attributes. The results are shown in figure 5of the three tests done for the standardization of clove enriched carrot cookies, in terms of their flavor, aroma, texture, color, taste and overall acceptability. The purpose of the standardization is to obtain a replication model for the study.

(Venkata Satish Kuchi, 2017). Any changes made by the panelist in first sensory evaluation were incorporated in the production before the second sensory evaluation. Hence, a standardized and replicable model was developed for future research and assistance.

The nutritional composition of a 3.5oz carrot cookie as control and 3.5oz carrot cookie containing 2.5g of clove as experimental is shown in table 2. The addition of clove in the cookies had a considerable increase in fiber, protein, folate and niacin. The manganese content raised from 4% to 100% having a very prominent contribution, proving that clove enriched carrot cookies are a good source of manganese and can contribute in bone health, regulation of blood glucose levels and play a part being an antioxidant.

Table 3 records the proximate analysis of the approved biscuits during sensory evaluation and the control biscuit which is without the addition of clove. The moisture content of the cookies increased from 13.6% to 18.20% owing to the high moisture content of carrot flour. Similar results were reported in the study conducted by (Joel Ndife, 2014) where moisture content of carrot flour was higher than the all-purpose flour. This moisture range could be a demerit as most microorganisms can thrive and survive in this moisture, leading to spoilage and deterioration. There was a significant increase in the protein content of the cookies from 6.60% to 9.46%. The increase is due to the substitution of clove in cookies. Other studies about incorporation of clove into food products have also reported an increase in protein content in cookies owing to the supplementation of clove (Neveen Fahmy Mohamed Agamy, Neveen Ahmed Alwardany, 2015). The result implies that the cookie samples were high in protein content and could be used as alternative protein source in protein deficiency among children who are the major consumers. The fat content increased from 12.15% to 13.50% in the cookies produced from carrot flour and enriched with clove owing to the aromatic oils of the clove. This will have a considerable effect on the shelf stability of cookies. The ash content of the cookies increased a little by 3.14 to 3.36% due to the presence of clove. The crude fiber showed an increase in the clove enriched cookies than an un-enriched one from 4.10% to 5.80% as both carrots and clove contain a considerable amount of fiber. The fiber content increased with the addition of carrot flour which are good sources of fiber. Crude fiber is known to aid the digestive system of human. This is an advantage as it helps in bowel movement and easy digestibility. Similar results were reported in the study by (Joel Ndife, 2014) where the use of clove was used to increase the fiber content of the food product. The ash content was also enhanced with clove enrichment in cookies from 3.14% to 3.36% because cloves are naturally high in ash. Ash is a non-organic compound containing mineral content of food and nutritionally it aids in the metabolism of other organic compounds such as fat and carbohydrate. Beta carotene level was constant for both control and experimental product; 8.332mg per 3.5oz of one cookie.

The increased fiber content of cookies will have several health benefits as it will aid in digestion and reduce the burden in colon and affect the gut health preventing from constipation. The crude fiber content of cookies was within the range of recommendation of not more than 6g/day and other nonabsorbable carbohydrates per 100g dry weight (Hsi-YangTang, 2020). The protein content was also higher with the addition of clove in the experimental product and this will help in building degenerative tissues, muscles and hormones of the body. Proteins are the building blocks of the body necessary for growth, repair and maintenance (Ignacio Echeverría, 2016).

4. Conclusion

Clove is a spice that contains bioactive compounds which provide therapeutic roles in cancer prevention, diabetes management, bone health, heart health, promote gut health and reducing oxidative stress. Cloves were incorporated in carrot cookies with two different carrot flour ratios, 50:50% and 70:30%. The Clove enriched carrot cookies had acceptable aroma, texture, color, taste and overall acceptability. Enriched cookies with clove were found to be nutritionally superior, owing to high protein and fiber content. Thus, the clove enriched cookies can conveniently be regarded as complete whole snack ideal for tea times. However, further research work should be focused on the shelf stability of the enriched cookies considering the high lipid content owing to aromatic oils of clove which can make the cookies prone to rancidity.

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6. Tables and Figures

Composite Flour	Carrot Flour	All Purpose Flour
CF1	50%	50%
CF2	70%	30%

Table 1: Treatment Plan for the Composite Flour

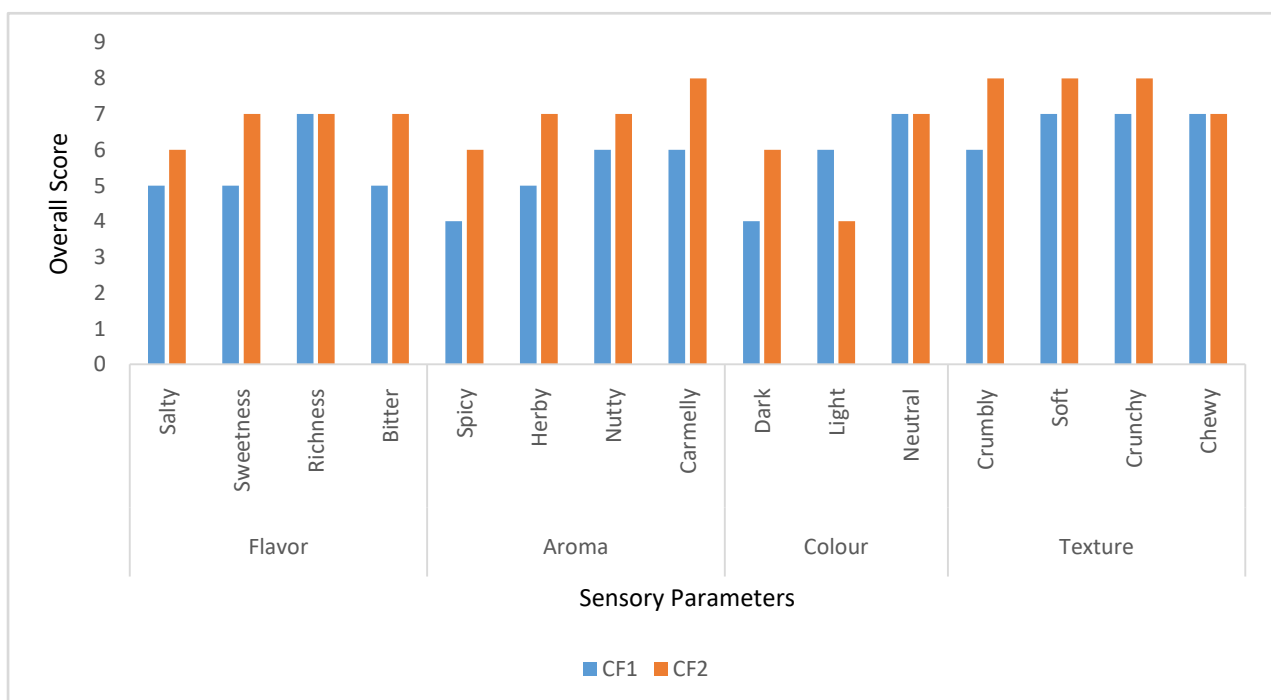


Figure 3: Sensory Evaluation of Clove Enriched Carrot Cookies

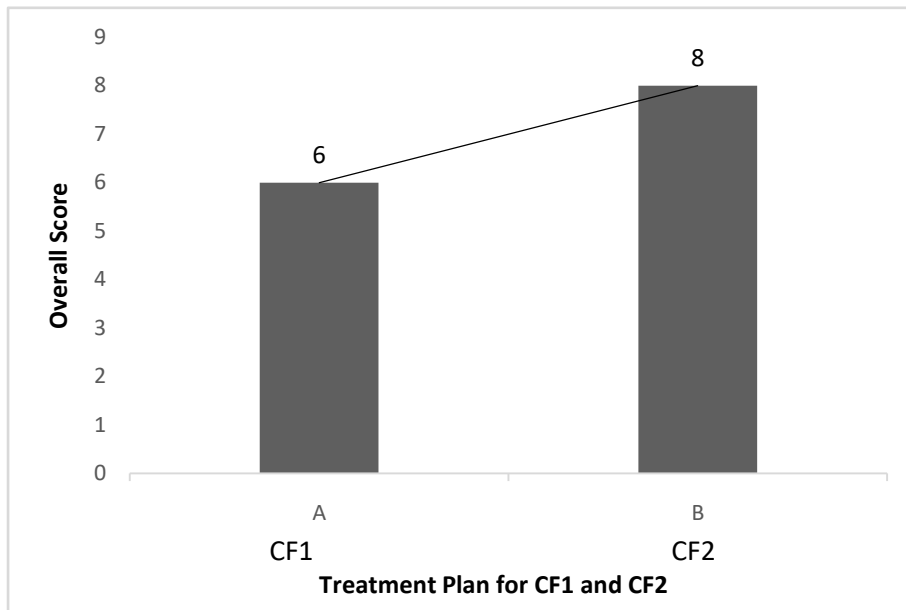


Figure 4: Overall Acceptability of Clove Enriched Carrot Cookies

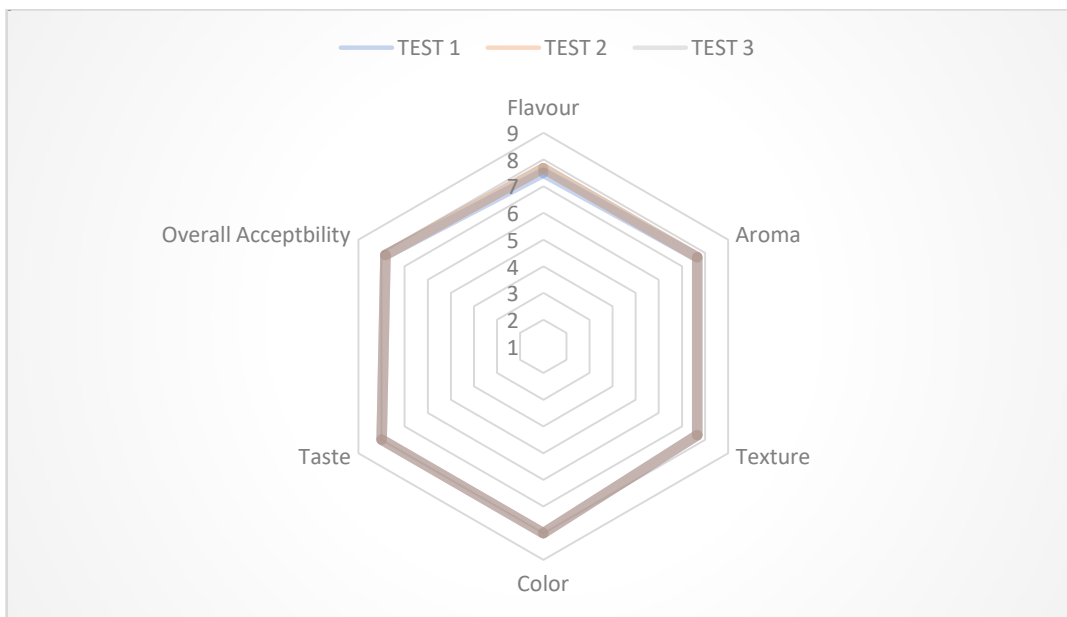


Figure 5: Standardization of Clove Enriched Carrot Cookies

Table 2: Nutritional Value for Clove Enriched Carrot Cookies

Composition	Amount per Cookies- Control	Amount per Cookie- Experimental
Calorie	110.9	117.9
Carbohydrates	14.1 g	15.5g
Dietary fiber	0.5 g	1g
sugars	0.4 g	0.45g
fats	5.6 g	5.9g
Saturated	0.6 g	0.7g
polyunsaturated	3.1 g	3.35g
monounsaturated	1.6 g	1.65g
proteins	1.4 g	1.65g
Vitamin A	23.4 %	23.6%
Vitamin B-6	0.9 %	1.5%
Vitamin B-12	0.1 %	0.1%
Vitamin C	0.8 %	3.8%
Vitamin D	0.0 %	0.0%
Vitamin E	0.2 %	1.2%
Calcium	2.8 %	4.1%
Copper	1.0 %	1.3%
Folate	5.0 %	5.75%
Iron	3.1 %	4.1%
Magnesium	0.9 %	2.4%
Manganese	4.3 %	100%
Niacin	3.4 %	3.4%
Pantothenic Acid	0.7 %	0.7%
Phosphorous	2.3 %	2.6%
Riboflavin	3.5 %	3.8%
Selenium	4.9 %	5.2%
Thiamin	5.6 %	5.6%
Zinc	0.7 %	0.7%
Beta Carotene	8.332 mg	8.332mg

Table 3: Proximate Analysis of Clove Enriched Carrot Cookies

No:	Parameters	Control	Experimental
1.	Moisture (%)	13.6	18.20
2.	Crude Protein (%)	6.60	9.46
3.	Crude Fiber (%)	4.10	5.80
4.	Crude Fat (%)	12.15	13.50
5.	Ash (%)	3.14	3.36
6.	NFE	74.01	67.88
7.	Beta Carotene	8.332mg	8.332mg

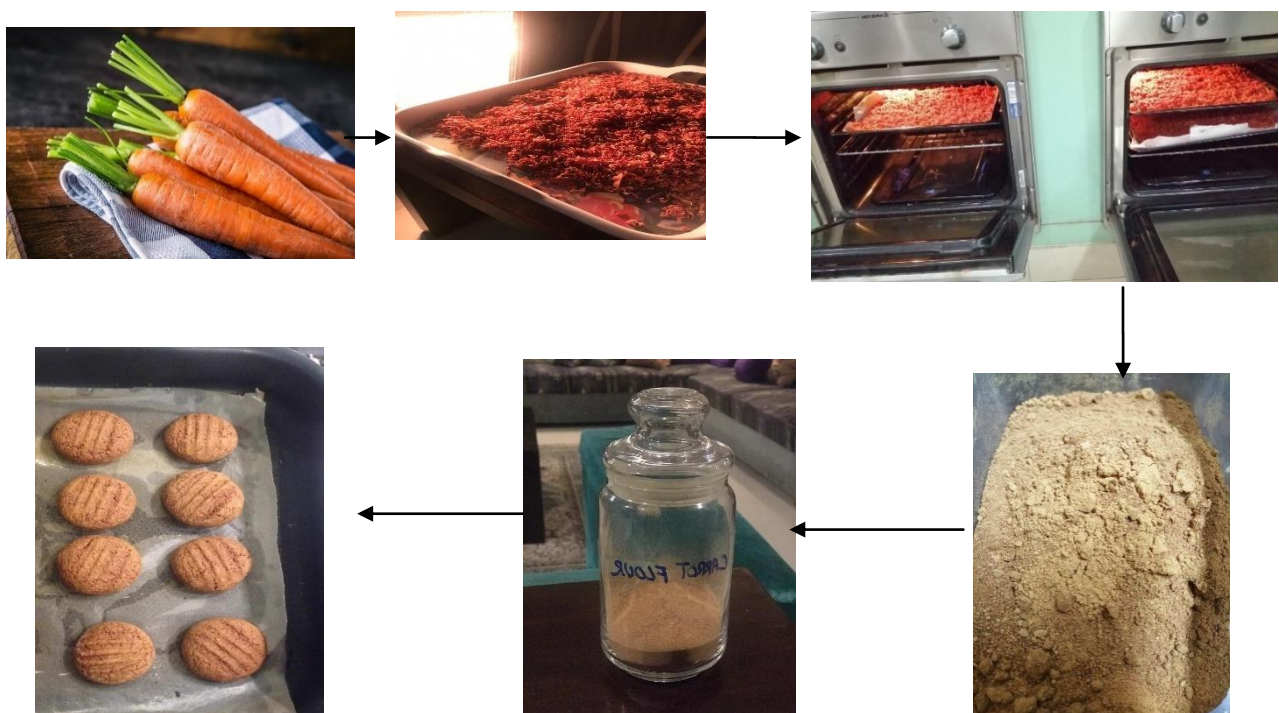


Figure 6: Development of Carrot Flour

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9. Competing Interests

The authors have declared that no competing interests exist.

10. Authors' Contributions

This work was carried out in collaboration among all authors. All authors contributed equally in various roles including formulation research goals, development of methodology, performing the experiments and analyzing data and writing the initial draft. The corresponding author coordinated the research activity as agreed by all authors. All authors read and approved the final manuscript.

Synthesis, Characterization, and *in Vitro* Antimicrobial Screening of Some Novel Heterocycles Linked to Tetrazolo [5,1-*f*]-1,2,4-Triazine Moiety

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ABSTRACT:

8-Hydrazinotetrazolo[5,1-*f*]-1,2,4-triazine (**1**) reacted with one or two carbon cyclizing reagents to yield various heterocyclic systems. Some of the representative members of the prepared compounds were screened for antimicrobial assessment.

Keywords: 8- Hydrazinotetrazolo[5,1-*f*]-1,2,4-triazine, heterocycles, *in Vitro* antimicrobial activity

INTRODUCTION

The tetrazole nucleus of several compounds has been received¹⁻¹¹ much great attention because of their wide range of therapeutic and biological activities.^{12,13} Compounds containing tetrazole core have diverse biological activities as antibacterial,^{1,3-9,14} antiproliferation,¹⁵ anticancer,¹⁵ and anticonvulsant¹⁶ agents. In view of the aforementioned facts, it seemed most interesting to synthesize some condensed or uncondensed 8-hydrazinotetrazolo[5,1-*f*]-1,2,4-triazine with the aim to evaluate their antimicrobial activities.

RESULTS AND DISCUSSION

Condensation of 8-hydrazinotetrazolo[5,1-*f*]-1,2,4-triazine (**1**) with equimolar amount of the appropriate aromatic namely : benzaldehyde, *p*-tolylbenzaldehyde, *p*-chlorobenzaldehyde, and *p*-nitrobenzaldehyde in boiling methanol afforded the corresponding 8-arylidenehydrazinotetrazolo[5,1-*f*]-1,2,4-triazines **2a-d** (Scheme 1), showing the expected NH in IR absorption as well as ¹H NMR signals characteristic of NH (D₂O-exchangeable), methylenic (-CH=N-), and aromatic protons. Their MS revealed the correct molecular ion peaks which were supported by elemental analyses. Subjecting these hydrazone derivatives **2a-d** to dehydrogenative cyclization with bromine in glacial acetic acid in the presence of anhydrous sodium acetate yielded the corresponding 8-aryl-1,2,4-triazolo[4,3-*d*]tetrazolo[5,1-*f*]-1,2,4-triazines **3a-d**. These structures lacked the NH absorption in IR and methylenic proton signal in ¹H NMR. In addition, the chemical proof for the assigned compounds **3a-d** were also prepared of hydrazine **1** with the equimolar amount of the corresponding aromatic acid chloride using phosphorus oxychloride through the unisolable aroylhydrazino – intermediates [A] which were formed and concomitantly dehydratively cyclized. The aforementioned products **3a-d** were proved to be identical in all respects (mp; mixed mp; TLC; and IR) with methods of cyclization mentioned above this article. Recently¹, the products **3a-d** gave directly by the reaction of 8-chloro tetrazolo[5,1-*f*]-1,2,4-triazine with corresponding of aromatic acid hydrazides. Elemental and spectra data of these products are consistent with the structure assigned above two cyclization reactions.

Refluxing of hydrazine **1** with excess of ethyl chloroformate in pyridine afforded a product, which showed neither ester-carbonyl absorption in IR nor ethyl group signal in ¹H NMR. None of the possible intermediate [B] (Scheme 1) was isolated but showed NH and CON absorptions in IR and was consequently, assigned the structure 1,2,4-triazolo [4,3-*d*]tetrazolo[5,1-*f*]-1,2,4-triazin-8-(9H)-one (**4**)

Condensation of cyclic amidrazone **1** with pyruvic acid at ambient temperature or heating at 100°C resulted in the corresponding hydrazone **5a**, which possessed IR characteristic of OH, NH, and CO absorptions. Similarly, ethyl pyruvate reacted with the hydrazine **1** to furnish the corresponding hydrazone **5b**, ¹H NMR spectrum of the latter contained the quartet and triplet pattern signals characteristic of ethyl group (*cf.* Experimental). Acid-induced heterocyclization of **5a** or **5b** (Scheme 1) by heating under reflux in acetic acid provided one and the same product, which displayed the disappearance of OH and NH absorptions but showed a CON absorption in IR region. The ¹H NMR spectrum of this cyclization product revealed no ethyl pattern. These data together with correct elemental analysis are compatible the structure 9-methyltetrazolo [5,1-*f*]-1,2,4-triazino[4,3-*d*]-1,2,4-triazin-8-one (**6**). The mass spectrum of **6** showed a peak corresponding to its

molecular ion at $m/z = 204$ ($C_6H_4N_8O$).

Condensative cyclization of **1** with equimolar amount of diethyl oxalate (Scheme 2) furnished the corresponding tetrazolo[5,1-*f*]-1,2,4-triazino[4,3-*d*]-1,2,4-triazine-8,9-(10H)-dione (**7**). Assignment of the latter structure and exclusion the possible non-isolable intermediate [**C**] was established by correct elemental analysis as well as the absence of quartet-triplet pattern of 1H NMR signals characteristic an ethyl group. Also, the mass spectrum of compound **7** caused molecular ion peak ($m/z = 206$) in agreement with its molecular formula ($C_5H_2N_8O_2$).

Furthermore, Condensation of the hydrazine **1** with acetylacetone (Scheme 2) was heated under reflux yielded the corresponding hydrazone derivative **8a** which showed IR absorptions characteristic of NH and CO, and 1H NMR spectrum of the latter product revealed the presence of NH (D_2O -exchangeable), methylene and two methyl group signals. Heating of **8a** with glacial acetic acid resulted in the cyclization to the 8-(3,5-dimethylpyrazol-1-yl) tetrazolo[5,1-*f*]-1,2,4-triazine system (**9a**) which revealed only C=N absorption and lacked NH and CO absorptions characteristic of the parent hydrazone **8a**, and appeared pyrazolyl CH proton signal in the 1H NMR spectrum.

Likewise, condensation of ethyl acetoacetate with cyclic amidrazone **1** (Scheme 2) provided formation the isoable hydrazone intermediate **8b**, which underwent base catalyzed cyclization upon refluxing with 0.1M sodium ethoxide to build either the pyrazolyl derivative **9b** or 8-methyl -1,2,4-triazolo[4,3-*d*]tetrazolo[5,1-*f*]-1,2,4-triazine structure (**10**, Scheme 2) according to a reported⁵ result which synthesized *via* the reaction of **1** with acetic acid. Structures elucidation of **9b** or **10** were based through the elimination an ethyl alcohol or an ethyl acetate molecule, respectively, from **8b** compound. Thus, the evidences of the cyclization of the hydrazone **8b** are: (a) the melting point and thin layer chromatography of the performed cyclization product are not similar to the structure **10**⁵ (mp 219-221°C); **9b** (mp 235-237°C) and (b) spectroscopic data of the product **9b** showed OH and lacked any amide absorption bands in the IR region; 1H NMR exhibited OH (D_2O -exchangeable) and pyrazolyl CH proton signals. Accordingly, the product was decisively assigned as the 8-(5-hydroxy-3-methylpyrazol-1-yl)tetrazolo[5,1-*f*]-1,2,4-triazine (**9b**) and formation the structure **10** could be excluded thereby. This is in accordance with the previous reports,^{17,18} but contradict another¹⁹ one on the reaction of ethyl acetoacetate with different cyclic amidrazones.

EXPERIMENTAL

General

Melting points were measured with a Gallenkamp apparatus and are uncorrected. The reactions were followed up and the purification of products was carried out on pre-(layer thickness 0.25mm; coated TLC plates Silica Gel-Merck), visualizin the spots in Iodine. IR spectra were recorded (KBr) on a Shimadzu FT-IR 8101 PC infrared spectrophotometer. The 1H NMR spectra were determined in $DMSO(d_6)$ at 300 MHz on a Varian Mercury VX 300 NMR spectrometer and their chemical shifts (δ/ppm) are reported using TMS as internal standard. Mass spectra were recorded on a HP model MS 5988 spectrometer at electron ionizing energy of 70 ev. Microanalyses were performed by the Microanalytical Unit, Cairo University, Egypt; the obtained results agreed satisfactorily with the calculated values.

Synthesis of 8-arylidenehydrazinotetrazolo[5,1-f]-1,2,4-triazines 2a-d (General Procedure)

A solution of 6 mmol of **1** in 15 cm³ methanol was added to 6 mmol appropriate aromatic aldehyde and the mixture was heated at 100°C for 10 min. The reaction mixture was kept at ambient temperature for overnight and the product which separated was filtered off, washed with ether, dried, and crystallized from methanol. The physico-chemical and spectra data of **2a-d** the following:

8-Benzylidenehydrazinotetrazolo[5,1-f]-1,2,4-triazine (2a, C₁₀H₈N₈)

Yield: 1.32g (83.54%); pale yellow; mp 158-160°C; **IR**: $\gamma=3325$ (NH), 1625 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): $\delta=11.60$ (s, 1H, D₂O-exchangeable NH), 8.20-7.90 (m, 5H, Ar H), 7.61 (s, 1H, methylenic H) 5.90 (s, 1H, CH) ppm; **MS**: m/z (%) = 240 (M⁺, 15), 241 (M⁺+1, 18).

8-p-Tolylmethylidenehydrazinotetrazolo[5,1-f]-1,2,4-triazine (2b, C₁₁H₁₀N₈)

Yield: 1.42g (83.03%); yellow; mp 160-162°C; **IR**: $\gamma=3340$ (NH), 1610 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): $\delta=10.87$ (s, 1H, D₂O-exchangeable NH), 8.17-7.82 (m, 4H, Ar H), 7.71 (s, 1H, methylenic H), 5.89 (2, 1H, CH), 2.30 (s, 3H, CH₃) ppm; **MS**: m/z (%) = 254 (M⁺, 30).

8-p-Chlorobenzylidenehydrazinotetrazolo[5,1-f]-1,2,4-triazine (2c, C₁₀H₇ClN₈)

Yield: 1.42g (78.45%); yellow; mp 170-172°C; **IR**: $\gamma=3350$ (NH), 1610 (C=N) cm⁻¹ **MS**: m/z (%) = 275 (M⁺, 16).

8-p-Nitrobenzylidenehydrazinotetrazolo[5,1-f]-1,2,4-triazine (2d, C₁₀H₇N₉O₂)

Yield: 1.52g (81.07%); orange; mp 190-192°C; **IR**: $\gamma=3340$ (NH), 1630 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): $\delta=10.68$ (s, 1H, D₂O-exchangeable NH), 8.31-7.74 (m, 4H, Ar H), 7.22 (s, 1H, methylenic H), 5.75 (s, 1H, CH) ppm; **MS**: m/z (%) = 285 (M⁺, 20).

Synthesis of 8-aryl-1,2,4-triazolo[4,3-d]tetrazolo[5,1-f]-1,2,4-triazine 3a-d (General Procedure)

Method A. To a solution of 4 mmol of the respective hydrazone **2a-d** in 15 cm³ glacial acetic acid containing 4 mmol bromine in 10 cm³ glacial acetic acid were added gradually with stirring. The reaction mixture was then warmed on a boiling water-bath for 5 min, left to cool and then poured onto water. The precipitated solid was filtered off, washed thoroughly with water, and crystallized from methanol.

Method B. A mixture of hydrazine **1** (6 mmol), particular aromatic acid chloride (6 mmol), and 10 cm³ phosphorus oxychloride was refluxed for 1h, then cooled and poured onto of 30 cm³ cold saturated solution of sodium bicarbonate. The crude solid that precipitated was filtered off, washed with water, dried, and finally crystallized from methanol.

Method C¹. A mixture of **1** (6 mmol) and appropriate aromatic acid hydrazide in ethanol (30 cm³) was refluxed for 3h, after cooling the mass product was filtered off and recrystallized from abs. ethanol.

The aforementioned methods **A**, **B**, and **C** are compatible with the assigned products **3a-d**.

1,2,4-Triazolo[4,3-d]tetrazolo[5,1-f]-1,2,4-triazin- 8-(9H)-one (4, C₄H₂N₈O)

A suspension of **1** (6 mmol) in 2 cm³ pyridine was treated with excess of ethyl chloroformate and the mixture was treated under reflux for 3h. The reaction mixture was poured onto ice-water and the product which separated was filtered off, washed with water, and crystallized from methanol. Yield: 0.85g (72.65%); mp 210°C; **IR**: ν =3300 (NH), 1690 (CON), 1625 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): δ =11.85 (s, 1H, D₂O-exchangeable), 5.75 (s, 1H, CH) ppm; **MS**: m/z (%) = 178 (M⁺, 26).

Synthesis of **5a** and **5b** (General Procedure)

To a solution of **1** (6 mmol) in 10 cm³ methanol, 6 mmol pyruvic acid or ethyl pyruvate were added and the mixture was kept at ambient temperature for 24h or heated at reflux for 1h. The product which separated was filtered off, washed with ether, and crystallized from methanol to provide **5a** and **5b**.

Pyruvic acid {tetrazolo[5,1-f]-1,2,4-triazin-8-yl} hydrazone (**5a**, C₆H₆N₈O₂)

Yield: 0.92g (68.49%); mp 175°C; **IR**: ν =3450 (OH), 3225 (NH), 1715 (C=O), 1625 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): δ =12.52 (s, 1H, D₂O-exchangeable OH), 11.84(s, 1H, D₂O-exchangeable NH), 5.90 (s, 1H, CH), 2.50(s, 3H, CH₃) ppm; **MS**: m/z (%) = 223 (M⁺+1, 27).

Ethyl pyruvate {tetrazolo[5,1-f]-1,2,4-triazin-8-yl} hydrazone (**5b**, C₈H₁₀N₈O₂)

Yield: 1.31g (79.39%); mp 185°C; **IR**: ν =3210 (NH), 1730 (C=O), 1600 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): δ =11.61 (s, 1H, D₂O-exchangeable NH), 5.85 (s, 1H, CH), 3.85 (q, 2H, CH₂CH₃), 2.55 (s, 3H, CH₃), 1.35 (t, 3H, CH₂CH₃) ppm; **MS**: m/z (%) = 250 (M⁺, 30).

9-Methyltetrazolo [5,1-f]-1,2,4-triazino [4,3-d]-1,2,4-triazin-8-one (**6**, C₆H₄N₈O)

A mixture of **5a** or **6b** (4 mmol) and 10 cm³ acetic acid was heated under reflux for 2h and then evaporated to dryness. The obtained residue was crystallized from methanol. Yield: 0.61g (66.30%); mp 220°C; **IR**: ν =1690 (CON), 1620 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): δ =5.90 (s, 1H, CH), 2.60 (s, 3H, CH₃) ppm; **MS**: m/z (%) = 204 (M⁺, 33).

Tetrazolo [5,1-f]-1,2,4-triazino [4,3-d]-1,2,4-triazin-8,9-(10 H)-dione (**7**, C₅H₂N₈O₂)

A mixture of **1** (6 mmol) and diethyl oxalate (6 mmol) was heated for 1h 100°C. after attaining room temperature, the mixture was triturated with methanol and the product which separated was filtered off and crystallized from methanol. Yield: 0.93g (68.38%); mp 250°C; **IR**: ν =3325 (NH), 1690, 1660 (CON), 1590 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): δ =12.10 (s, 1H, D₂O-exchangeable NH), 5.75 (s, 1H, CH) ppm; **MS**: m/z (%) = 206 (M⁺, 19).

Synthesis of **8a** and **8b** (General Procedure)

To a solution of **1** (6 mmol) in 15 cm³ methanol was added to 6 mmol acetylacetone or ethyl acetoacetate and the mixture was heated under reflux for 2h. The separated product was filtered off, washed with ether and crystallized from methanol to perform **8a** and **8b**.

Acetylacetone {tetrazolo[5,1-f]-1,2,4-triazin-8-yl} hydrazone (**8a**, C₈H₁₀N₈O)

Yield: 1.13g (73.38%); mp 175°C; **IR**: γ =3240 (NH), 1700 (C=O), 1625 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): δ =11.85 (s, 1H, D₂O-exchangeable NH), 5.80 (s, 1H, CH), 4.12 (s, 2H, CH₂), 2.35, 2.20 (2s, 3H each, 2CH₃) ppm; **MS**: m/z (%) = 234 (M⁺, 18).

8-(3,5-Dimethylpyrazol-1-yl) tetrazolo[5,1-f]-1,2,4-triazine (**9a**, C₈H₈N₈)

A solution of **8a** (4 mmol) in 10 cm³ glacial acetic acid was heated under reflux for 2h and then evaporated dryness under reduced pressure. The obtained residue was crystallized from methanol; Yield: 0.53g (57.61%); mp 195-197°C; **IR**: γ =1625 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): δ =5.75 (s, 1H, CH), 5.25 (2, 1H, pyrazolyl CH), 2.30, 2.25 (2s, 3H each, 2CH₃) ppm ; **MS**: m/z (%) = 216 (M⁺, 7).

Ethyl acetoacetate {tetrazolo[5,1-f]-1,2,4-triazin-8-yl} hydrazone (**8b**, C₉H₁₂N₈O₂)

Yield: 1.22g (70.12%); mp 190-192°C; **IR**: γ =3295(NH), 1735 (C=O), 1615 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): δ =12.15 (s, 1H, D₂O-exchangeable NH), 5.78 (s, 1H, CH), 4.20 (q, 2H, CH₂CH₃) 4.10 (s, 2H, CH₂), 2.40 (s, 3H, CH₃), 1.28 (t, 3H, CH₂CH₃) ppm ; **MS**: m/z (%) = 265 (M⁺+1, 8).

8-(5-Hydroxy-3-methylpyrazol-1-yl) tetrazolo[5,1-f]-1,2,4-triazine (**9b**, C₇H₆N₈O)

A solution of **8b** (3 mmol) in 15 cm³ freshly prepared 0.1M sodium ethoxide was heated under reflux for 2h. The resulting solution was neutralized with acetic acid and product which separated was filtered off and crystallized from methanol; Yield: 0.54g (65.85%); mp 235-237°C; **IR**: γ =3400 (OH), 1615 (C=N) cm⁻¹; **¹H NMR** (DMSO-d₆): δ =12.35 (s, 1H, D₂O-exchangeable OH), 5.80 (s, 1H, CH), 5.30, (s, 1H, pyrazolyl CH), 2.35 (s, 3H, CH₃) ppm ; **MS**: m/z (%) = 218 (M⁺, 30).

ANTIMICROBIAL ACTIVITY

The antimicrobial activity of some the synthesized compounds was determined *in vitro* against a variety of bacteria. The tests were carried out using disc diffusion method^{20,21} against *Gram*-positive bacteria and *Gram*-negative bacteria were dissolved in *DMF*, and activity mentioned on 1000 ppm. Agar plates were surface inoculated uniformly from fresh broth culture of the *Gram* bacteria. The discs were incubated at 25°C for 1h to permit good diffusion then incubated at 28°C for 24h, and the zones of inhibition were measured and displayed in **Table** .

Table: Antimicrobial activity of some synthesized compounds

Test compound	Actibacterial activity			
	Gram +ve bacteria		Gram-ve bacteria	
	<i>Baillus subtilis</i>	<i>Streptococci</i>	<i>Klebsiella Pneumoniea</i>	<i>Escherechia Coli</i>
2a	++	++	+	+
2b	+	+	++	++
2d	+++	+++	++	+++
3a	+	+	-	+
3b	++	++	++	++
3d	+++	+++	++	+++
4	+	+	-	-
5a	+	+	+	+
5b	++	++	++	++
6	+	-	-	-
7	-	-	+	+
8a	++	++	+	-
8b	+++	+++	-	-
9a	+++	+++	+++	+++
9b	-	-	+	+
Control	-	-	-	-
DMF				

The data obtained from above table indicate the compounds **2d**, **3d**, **8b**, and **9a** are higher activity but **5b**, **8a** are moderate activity and some of compound exhibit lower or no activity against *Gram* bacteria.

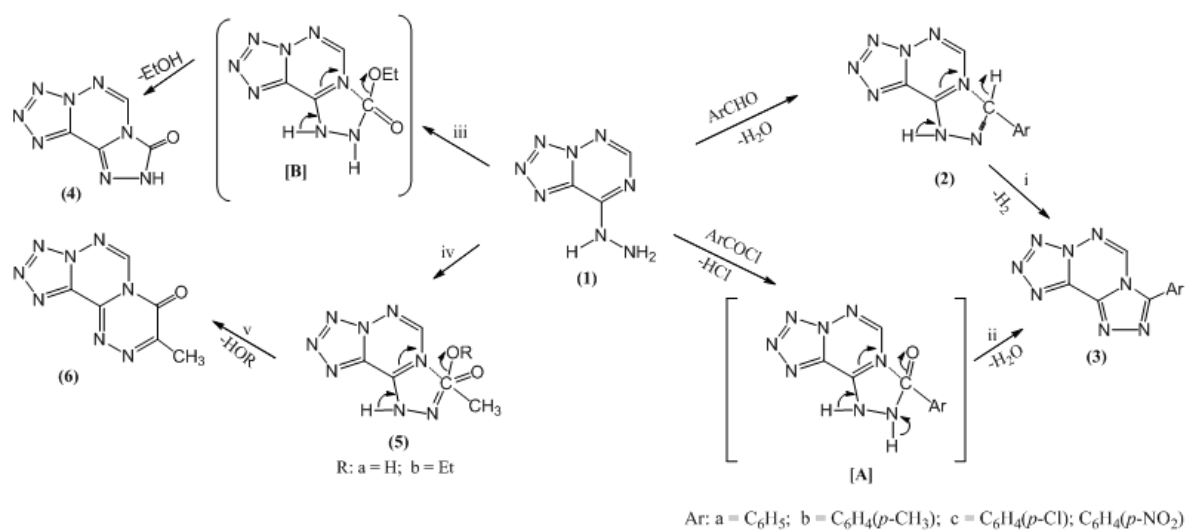
CONCLUSION

In this article we reported the synthesis of some novel heterocycles starting from 8-hydrazinotetrazolo[5,1-*f*]-1,2,4-triazine (**1**). Investigation of their antimicrobial activity revealed that **2d**, **3d**, **8b**, and **9a** were the most active compounds although the activity was significantly less than that of the positive control.

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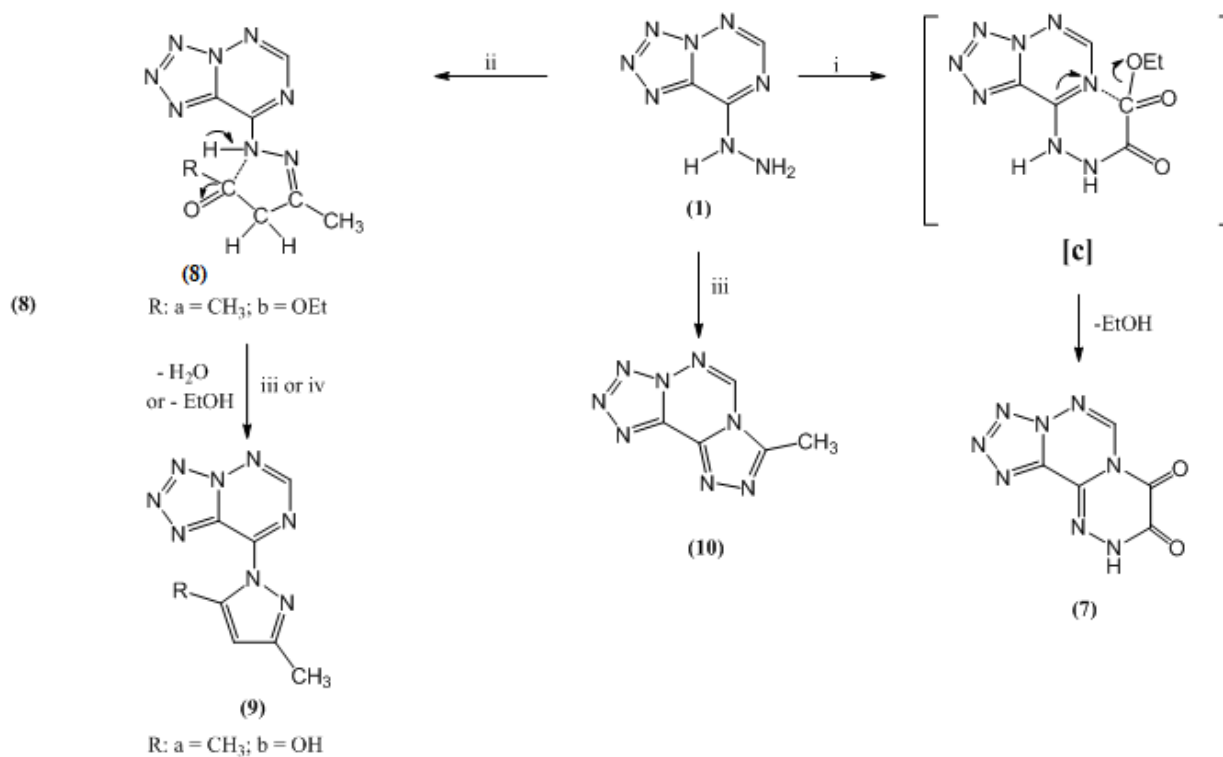
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Reagents: i, Br₂/AcOH; ii, POCl₃; iii, ClCOOEt; iv, CH₃COCOOR; v, AcOH

Scheme 1

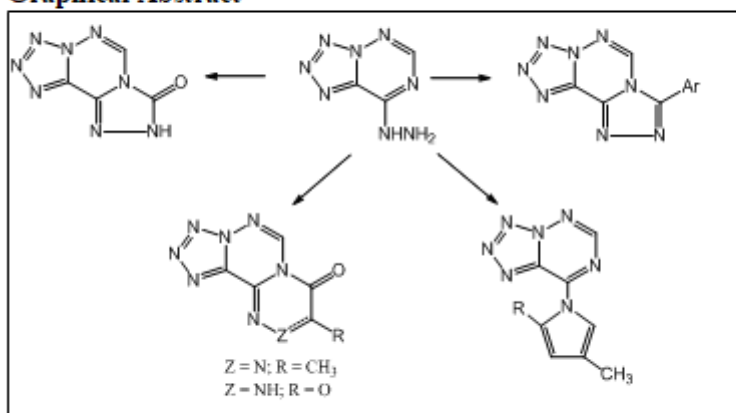


Reagents: i, (COOEt)₂; ii, CH₃COCH₂COR; iii, AcOH; iv, NaOEt

Scheme 2

Running Title: Tetrazolo[5,1-f]-1,2,4-triazine

Graphical Abstract



Use of Rice Husks as partial replacement of coarse aggregates in concrete paving blocks

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Abstract

Concrete paver blocks were first introduced in Holland in the fifties as replacement of paver bricks which had become scarce due to the post-war building construction boom. These blocks were rectangular in shape and had more or less the same size as the bricks. The blocks are made of cement, sand, and ballast with water.

Paving blocks are widely used in Kenya in residential and commercial areas. They can be used in areas with light or heavy traffic. They come in different sizes and shapes as per structural and client requirements. Rice husks are a byproduct of rice which is widely grown in parts of Central and Western Kenya. Rice husks disposal is a big challenge in rice processing companies with only a handful of it being used by cement manufacturing companies in their boilers.

In order to find alternative use of rice husks, this study was carried out to investigate its suitability for use in paving blocks as partial replacement of coarse aggregates. Seven replacement ratios were used viz: 5%, 10%, 15%, 25%, 50%, 75% and 100%. The samples were tested for compressive strength, tensile splitting strength and water absorption. It was found that, at 5% replacement rate, paving blocks had compressive strength of 65.61MPa which is suitable for use in high traffic areas. In addition, 10% and 25% replacement rate produced paving blocks suitable for use in medium traffic areas with compressive strength of 46.86MPa, 45.08MPa respectively.

Key words: rice husks, compressive strength, tensile splitting test, water absorption.

1. INTRODUCTION

Paving blocks are concrete products that are easy to manufacture and at any location as long as there is adequate space and water. This has made the use of paving blocks widespread. They are versatile, aesthetically attractive, functional and cost effective and require little or no maintenance if correctly manufactured and laid (B. Shanmugavalli et al., 2017).

Concrete paver blocks were first introduced in Holland in the fifties as replacement of paver bricks which had become scarce due to the post-war building construction boom. These blocks were rectangular in shape and had more or less the same size as the bricks. During the past five decades, the block shape has steadily evolved from non-interlocking to partially interlocking to fully interlocking to multiply interlocking shapes (Nataraja & Das, 2012)

The blocks are made of cement, sand, and ballast with water. To ensure the blocks are strong enough to carry vehicular loading, the mixing must be done in the right ratio and adequate curing for at least seven days. Paver block is, unreinforced pre-cast cement concrete paving units used in the surface course of pavement. They are high strength concrete mouldings in various shapes, sizes and colours (Pitroda et al., 2015).

Currently, the focal challenges facing the building and housing sector are highlighted and emphasized on the reduction of environmental impact. In this context, an environmentally and friendly sustainable materials using renewable and indigenous resources are in full development (Winarno, 2019). Agricultural waste disposal is a menace in most countries Kenya included. One of the agricultural wastes is rice husk with only a handful of the waste being used in boilers for cement manufacturing companies. Rice husks are agro-industrial by-products coming from rice hulling. Due to its low nutritional value, it is not considered appropriate for use as animal feed. The siliceous composition of rice husks makes it resistant to natural degradation which can produce large environmental load (Salas & Veras Castro, 1985).

Adequate alternative disposal arrangements must be considered to avoid environmental degradation. With the growing Kenyan population, demand for housing and commercial centers is equally increasing. This provides a large consumer for eco-friendly concrete and concrete products. This formed the basis of this research.

2. EXPERIMENTAL PROGRAM

2.1 Materials

2.1.1 Fine aggregates

River sand was purchased from local suppliers in Machakos town. It conforms with standards in BS 882 including, being clean, free of salts and other inorganic substances.

2.1.2 Coarse aggregates

Well graded coarse aggregates of sizes 5-12mm were purchased from local suppliers in Machakos town.

2.1.3 Cement

Portland cement N-32 was purchased locally in from suppliers in Machakos town. This is the most popular type of cement in Kenyas construction industry.

2.1.4 Water

Portable water from the University was used.

2.1.5 Rice husks

Rice Husks was purchased from Mwea Rice Growers Multipurpose Co-operative Society Ltd in Kirinyaga county.



Figure 1: Rice husks

3.0 MIX PROPORTIONS

The batching of materials was done using percentage by volume. The mix ratio was 1:0.5:1 (cement, sand and coarse aggregates). A control sample of M35 was prepared. Seven different samples were prepared at 5%, 10%, 15% 25%, 50%, 75% and 100% coarse aggregates replacement ratios. A constant water-cement ratio of 0.4 was used. The mix proportions are shown in the table 1.

Table 1: Mix proportions

S. No.	Description	Cement	Rice Husks	Fine Aggregates	Coarse Aggregates
1	C35	1	0	0.5	1
2	5% Replacement	1	0.05	0.5	0.95
3	10% Replacement	1	0.10	0.5	0.90
4	15% Replacement	1	0.15	0.5	0.85
5	25% Replacement	1	0.25	0.5	0.75
6	50% Replacement	1	0.50	0.5	0.50
7	75% Replacement	1	0.75	0.5	0.25
8	100% Replacement	1	1.00	0.5	0.00

All dry ingredients (cement, rice husks, fine aggregates and coarse aggregates) were carefully measured and poured in a concrete mixer where they were mixed for 3 minutes. Water was then added and mixing continued for five minutes. Due to the small volume of the concrete, it was further hand mixed in a mixing tray for two minutes. Total mixing time was ten minutes.

4. PRODUCTION OF PAVING BLOCKS

Concrete mixture was poured in a semi-automated moulding machine and well distributed. It was then vibrated for three minutes to ensure that no void spaces within the mould and to remove air bubbles present within the mix. The loaded timber mould was then transferred to a flat surface and allowed to dry for 24hrs. The demolded pavers were put in curing containers for stipulated time of 7,14,21 and 28 days. The produced concrete blocks were rectangular in shape and measured 60mm*100mm*200mm



Fig 2: Semi-automated moulding machine

5. TESTS

A. Compressive strength test

In order to study the strength development in concrete, compressive strength tests were conducted at the ages of 7, 14, 21 and 28 days.

Compressive strength (N/mm²)= Load at failure/area of the sample



Figure 3: Compressive strength test.

B. Split tensile test

After 28 days of curing, the samples were tested for split tensile strength.



Fig 5: Split sample

$$T = \frac{2P}{\pi Ld}$$

Where: T= splitting tensile strength

P=maximum applied load indicated by testing machine

L=length

D=diameter

C. Water absorption

After 28 days of curing, samples were tested for water absorption. They were first weighed to get the wet weight, then placed in oven for 24 hrs. at 105°C after which they were weighed to get oven dry weight.

$$\text{Water absorption} = \frac{(WW - DW)}{DW} * 100$$

Where WW-wet weight

DW-dry weight after oven drying

6. RESULTS AND DISCUSSION

A. Compressive strength

Compressive strength development over time is shown in figure 5.

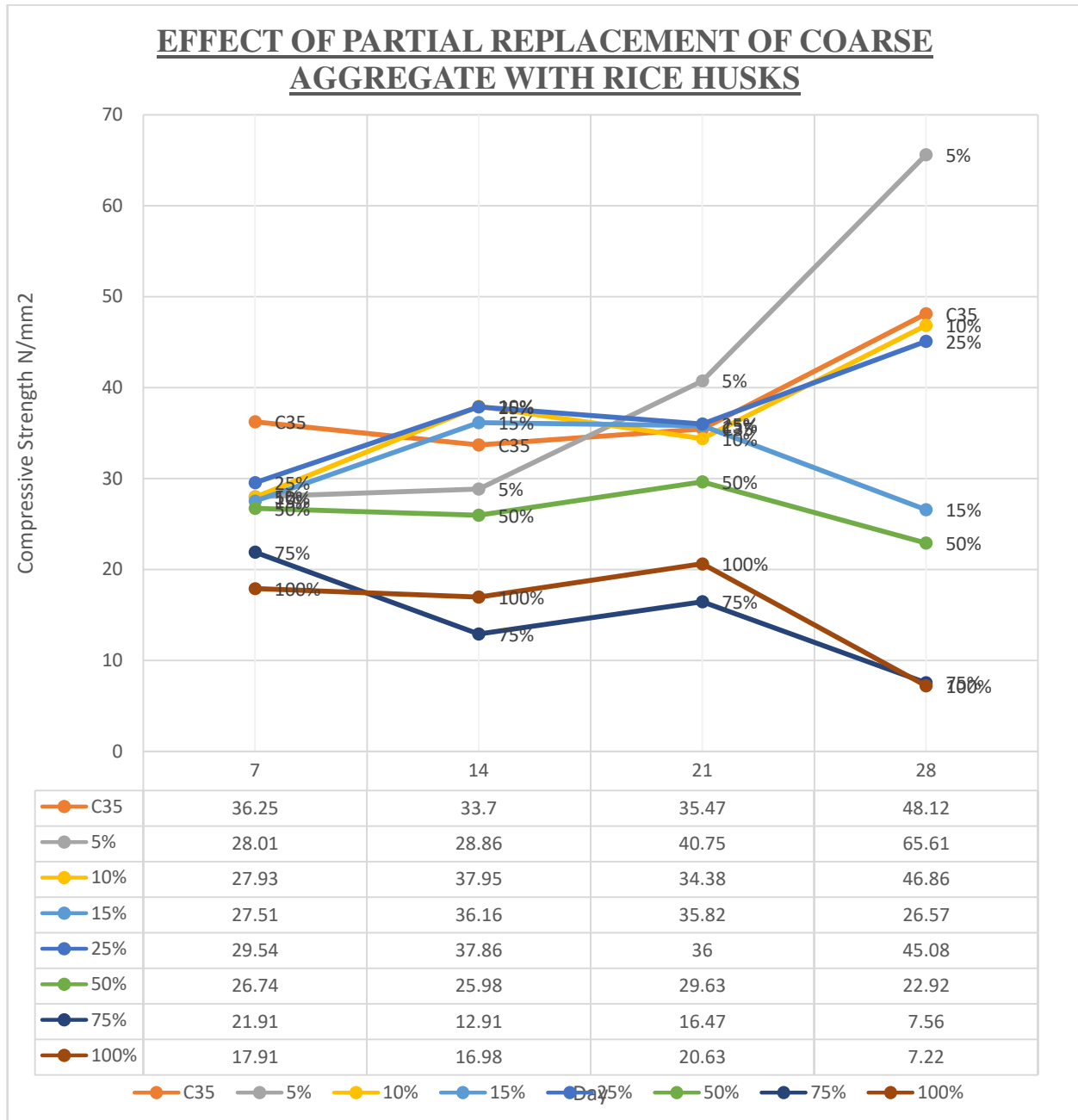
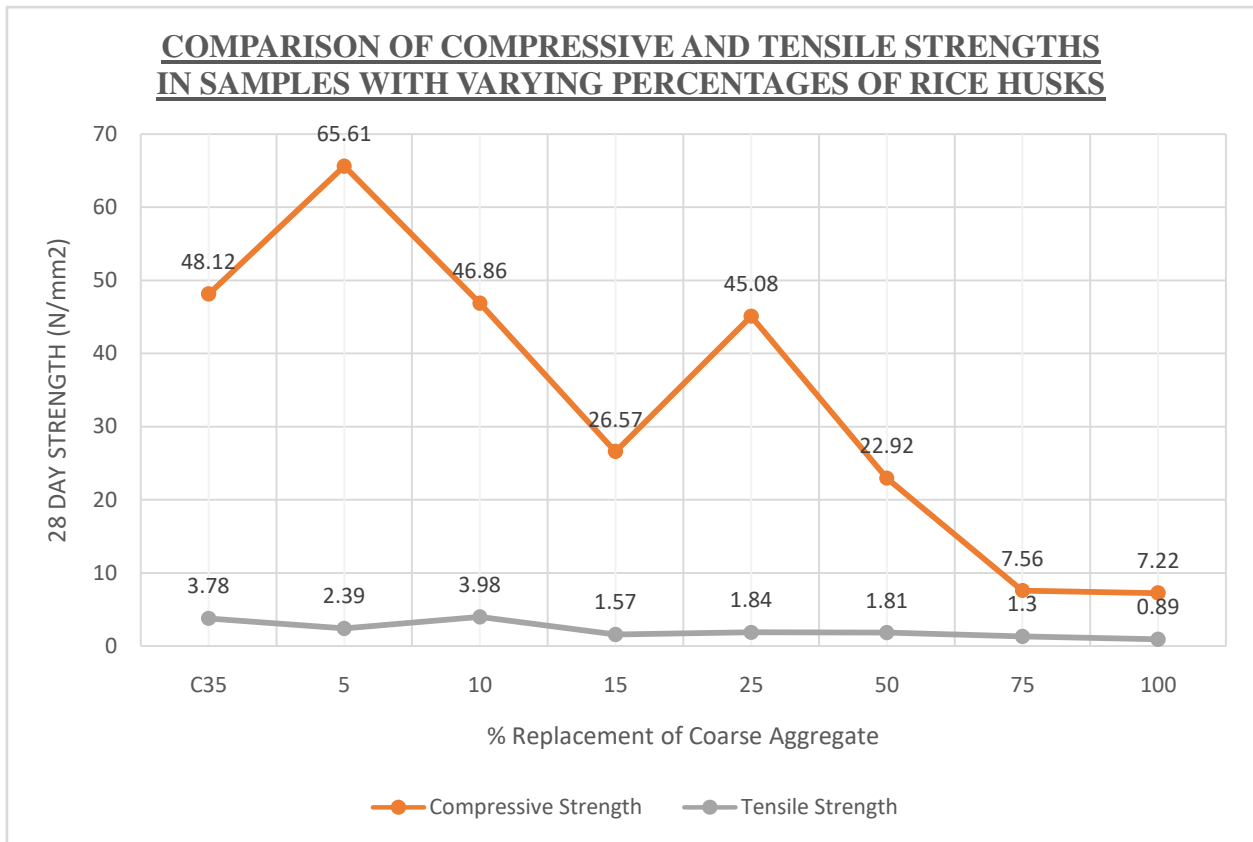


Fig 6: Partial replacement of coarse aggregates with rice husks.

At 5% replacement ratio of coarse aggregates, the concrete had the highest compressive strength of 65.61MPa. As the rate of replacement increased, ie, 10%, 15%, 25%, 50%, 75%, and 100% the compressive strength was 46.86MPa, 26.57MPa, 45.08MPa, 22.92MPa, 7.56MPa and 7.22MPa respectively. Paving blocks made with 5% coarse aggregates replacement ratio can be used to pave heavy traffic areas whilst those made with 10% replacement ratio can be used to pave medium traffic areas. Concrete made by rice husks provides an outstanding lightweight concrete because of

the interconnected network of porosity which characterizes these materials (Winarno, 2019). In such, lightweight materials are well suitable for use in earthquake prone areas.



B. Split tensile test

Fig 7: Tensile strength test

Tensile strength was seen to decrease with increase in rice husks content. similar trend was observed for compressive strength. However, at 5% replacement ratio, tensile strength was 2.39 N/mm² while the compressive strength was 65 MPa. When in use therefore, the paving blocks should be laid in such a manner that the loading is axial. The splitting tensile strength of concrete is mainly affected by the paste quality. The properties of aggregates affect the quality of paste and interfacial transition zone

C. Water absorption

Results for water absorption are shown in figure 8.

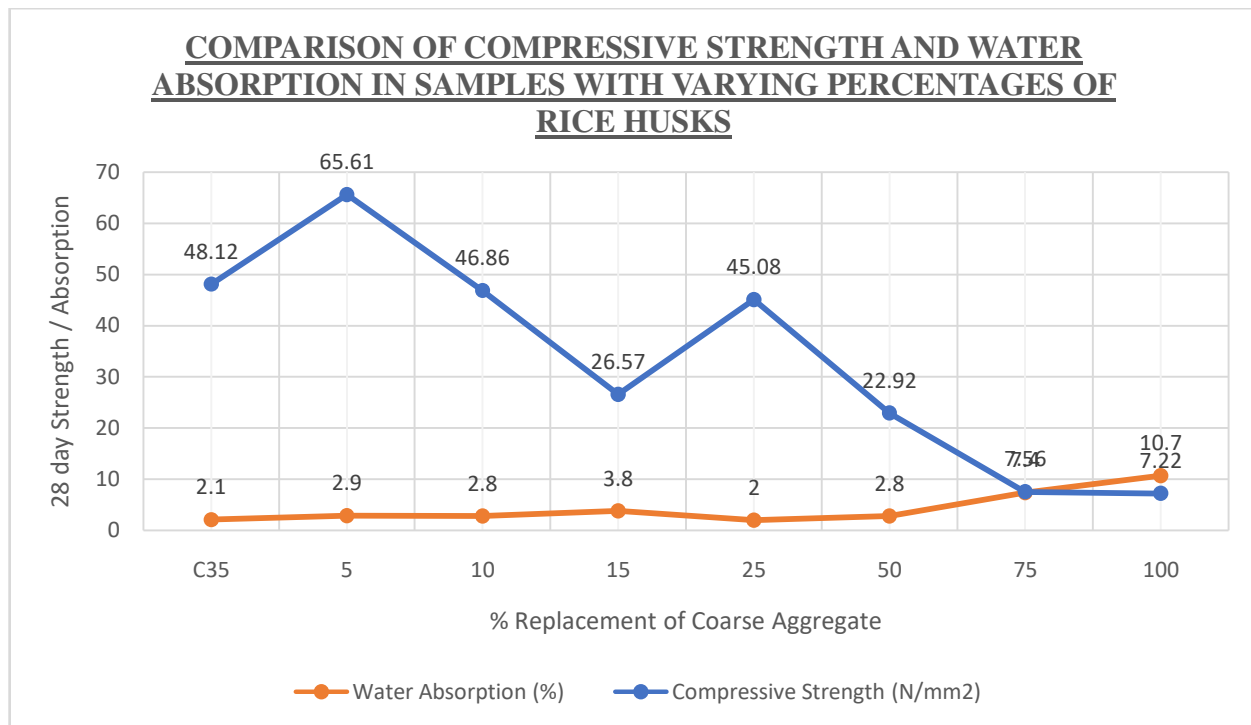


Fig 8: Rate of water absorption

Water absorption rate was varying for each replacement ratio. The highest water absorption rate was at 100% replacement rate at 10.7%. The rate of water absorption was inversely proportional to compressive strength. ASTM states that the average absorption of test samples shall not be greater than 5% with no individual unit greater than 7% (El Nouhy & Zeedan, 2012). Higher water absorption indicates a higher porosity in concrete (Meikandaan, 2016).

7. CONCLUSION

The results of this study show that, coarse aggregates can be replaced with rice husks. Paving blocks made with 5% rice husks in place of coarse aggregates have a compressive strength suitable for use in high traffic areas. In addition, with 10% and 25% replacement rates, the paving blocks can be used for medium traffic pavements. Due to the nature of rice husks, the resulting concrete is lightweight.

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