1. Introduction

India possesses a distinct identity for its geography, history, culture and great biodiversity. It ranks sixth among the twelve mega-biodiversity centers of the world and is home for an unusually large number of endemic species [1]. Unfortunately, much of the ancient knowledge and many valuable plants are being lost at an alarming rate. The continued commercial exploitation of these plants has resulted in receding the population of many species in their natural habitat. Vacuum is likely to occur in the supply of raw plant materials that are used extensively by the pharmaceutical industry as well as the traditional practitioners [2].

Diversity Act (2002) and Rule (2004) enforced the noble thought of protecting our biodiversity especially crude drugs from wild origin [3]. Therefore it has become an essential criterion for all the herbal industries and those working on medicinal plants, to produce these crude drugs by cultivation in the fields. A series of food scares and the controversy surrounding genetically modified crops have prompted heated debate about the safety and integrity of our food and herbal medicines. Increasing consciousness about conservation of environment as well as health hazards associated with agro-chemicals and consumer’s preference to safe and hazard-free food are the major factors that lead to the growing interest in alternate forms of agriculture in the world. Against this background, demand for organically grown food has been growing rapidly [4]. Organic agriculture is among the broad spectrum of productive methods that are supportive to the environment. The demand for organic...
food is steadily increasing both in the developed as well as developing countries with an annual average growth rate of 20–25% [5]. Until now this perception that, organically grown food and medicinal plants are ‘better for you’ appears to have been largely based on intuition rather than conclusive evidence. Cymbopogon citratus (DC) Stapf belongs to the family Poaceae and commonly known as ‘lemon grass’. C. citratus is one of the important sources of essential oils for flavour and fragrance industries worldwide. It is also greatly used in various traditional medicines as infusion or decoct. It contains active ingredients like tannins, saponins, flavonoids, alkaloid, phenols, and anthraquinones. Citral, geranial, nerol, myrcene, geraniol, linalool, tumerone, eugenol, isoeugenol, limonene, borneol, citronellol, nerol, α-terpineol, α-Caryophyllene are found in the essential oil [6,7]. The Cuban population has employed the species as an antihypertensive and anti-inflammatory drug [8]. In eastern Nigeria, this plant has been utilized for treating obesity, coronary disease and diabetes [9]. Therefore, in the present study, an attempt has been made to perform the toxicological and pharmacological profiling of organically (OCC) and non-organically (NCC) cultivated C. citratus.

2. Materials and methods

2.1. Cultivation and harvesting

The land for the organic treatment was selected and converted (period 2.5 years before 1st harvest) [10] and the parallel area with marked buffer zone was selected for conventional treatment. The germplasms were procured from Organic India Pvt. Ltd. Lucknow and cultivated by adopting the complete randomized statistical design in twelve replicates of each treatment. Two different treatments were utilized for organic and non-organic (in terms of fertilizer and insecticide/fungicide) [11,12]. Aerial parts of C. citratus were harvested at the time of maturity (inflorescence) and botanically authenticated from the Department of Botany, Rashtrasant Tukadoji Maharaj, Nagpur University, Nagpur. Voucher specimens (specimen no. 9787) have been deposited for future reference. The aerial parts were cut into small pieces and lyophilized (Lyodel-00-12, Chennai, India). The material was pulverized, again lyophilized and stored in air tight containers for further use.

2.2. Extraction and phytochemical screening

The decoctions were prepared by extracting the well dried crude powder of plant material (100 gm each) using an optimized method, with 1000 ml of Milli Q (MQ) water (BioAGE Direct Ultra, Punjab, India) at 80 °C for about 1 h with constant stirring. The aqueous portion was collected and the residual was extracted again with another 1000 ml of MQ water. The resulting cooled aqueous extracts were collected, combined, filtered by gauze, concentrated in vacuum (25 °C) and then lyophilized for drying [13]. The final yield of OCC and NCC, were 18.47, 20.15% w/w respectively. The extracts were each 2 ml eppendorf tubes.

2.3. Toxicological profiling using bioluminescent bacteria

This is the first time bioluminescent bacteria are utilized to check the level of toxicity in the plant extracts. A modified nutrient agar [17] was utilized for growing test organism, Vibrio harveyi. Preliminarily, bacteria mother culture of 50 ml was grown overnight at optimum temperature (20 °C) in an orbital shaker at 120 rpm and then 1 ml of overnight grown culture was dispensed in each 2 ml eppendorf tubes.

2.3.1. Qualitative estimation of luminescence

The stock solution (100 mg/ml) of plant extracts was added to the Eppendorf tubes containing bacteria culture, in a concentration range of 1–10 mg and highest concentration of 100 mg. Bacteria culture with plant extracts was incubated for 30 min, followed by streak on modified nutrient agar plates and finally incubated overnight at 20 °C. A rapid qualitative luminescence measurement was made by observing these plates in completely dark room. A 16 mega-pixel low light adjusted digital camera was stationed inside a self-made light proof box for capturing appropriate photographic evidences of luminescence on cultured plates [18].

2.3.2. Quantitative estimation of luminescence

For quantitative measurement, a Glomax- Promega Luminometer was used. The luminescence intensity was measured in terms of relative light units (RLU) for a series of plant extracts (1–10 mg and highest concentration of 100 mg) aided luminescent bacterial culture in test tubes after 30 min of incubation at 20 °C. To verify, whether the cells were affected by the toxic components present in plant extracts, their light output was compared with the light output of the control cells. The effective nominal concentrations (EC) resulting in 50% inhibition of luminescence were determined by plotting the percentage luminescence inhibition against concentration (mg/ml) and the results obtained for each system were compared by linear regression [19].

2.4. Pharmacological profiling

2.4.1. Animals

Swiss albino mice (20–25 g) of either sex obtained from animal house of Department of Pharmaceutical Sciences, R.T.M. Nagpur University, Nagpur, were used. The animals were fed a standard pellet diet and water ad libitum. They were maintained in a controlled environment and temperature (22 ± 5 °C with 12-h of light/dark cycle). All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), sanctioned protocol no. 25/PCSEA, dated 14 Jan 2013.

2.4.2. Acute toxicity and diabetes induction

Mice were divided into test and control groups (n = 3) and fixed oral dose as per OECD 420 guidelines were followed [20]. The basal body weight of animals was recorded and nicotinamide (NAD) (SRL, Mumbai, MS, India) (210 mg/kg b.w., i.p.), dissolved in saline, was injected intraperitoneally 15 min before administration of streptozotocin (STZ) (SRL, Mumbai, MS, India) (180 mg/kg b.w., i.p.), which was dissolved in citrate buffer (pH 4.5) immediately before use. Controls received both vehicles. After 72 h of streptozotocin injection, blood was withdrawn from overnight fasted animals and blood glucose level was assessed by glucometer. The mice with a blood glucose level above ≥180 mg/dL were selected for the experiment as diabetic mice. During the experimental period, the animals’ body weights were monitored once a week [21].

2.4.3. Acute anti-diabetic studies

The diabetic mice were fasted overnight and divided randomly in six groups of six mice each as follows: Diabetic/negative control-received vehicle (distilled water 10 ml/kg; p.o.); Positive control-received glibenclamide (10 mg/kg; p.o.); next four groups received OCC and NCC in a dose range of (100 and 200 mg/kg p.o.). One non-diabetic group was utilized as neo/normal control which received vehicle only. Post treatment, the blood samples were collected by snap-cut at the tip of the tail at 0 and 1.5th h (fasting). Later, all the animals were given food and water ad libitum and next samples were collected at 4th and 6th h (postprandial) and blood glucose
was measured by using commercially available glucometer (Accu-
Check, Roche, Germany).

2.4.4. Sub-chronic anti-diabetic studies

The diabetic animals and one normal control group were further
utilized to assess the sub-chronic anti-diabetic activity
and effects on body weight at 0, 7, 14 and 21st days. The blood
glucose was recorded by withdrawing blood samples by snip-cut
method at the tip of the tail and reported the results as mg/dL. On
22nd day, whole blood was withdrawn through retro-orbital vein
puncture of all groups by anaesthetizing the animals with i.p.
injection of thiopental sodium (half volume in eppendorf for
serum and half volume in EDTA sprinkled vacutainer tubes for
erythrocyte lysate) and finally all animals were sacrificed by cer-
vical dislocation and the pancreas and liver were removed for
further analysis [22].

2.4.5. Estimation of lipid profile and liver enzymes in blood serum of
diabetic mice

Blood samples were centrifuged within 6 h (stored at 8 °C) of
collection at 1000 rpm for 10 min and serum obtained was
immediately analyzed to determine the total cholesterol (TC), tri-
glycerides (TG), low-density lipoprotein (LDL), very low-density
lipoprotein (VLDL) levels and high-density lipoprotein (HDL) level
using semi — autoanalyzer (Semi-AutoAnalyzer, Microlab 300,
Merck) by using diagnostic kits and reagents obtained from Merck,
India. The serum samples were also examined for the estimation
of serum glutamate oxaloacetate transaminase (SGOT) level and
serum glutamate pyruvate transaminase (SGPT) [23].

2.4.6. In vivo antioxidant activity in erythrocyte lysate and liver

As per the results, only the control mice and higher dose
(200 mg/kg) treated groups were shortlisted for the further
analysis.

2.4.6.1. Lipid peroxidation (LPO) and catalase (CAT).
The procedure described by Kirana et al. was followed to estimate the lipid
peroxidation and catalase activity [24].

2.4.6.2. Superoxide dismutase (SOD). The concentration of SOD was
determined as per Joharapurkar et al. [25].

2.4.6.3. Glutathione reduced (GSH). The GSH was estimated using
the procedure described by Ghosh et al. [26].

2.4.6.4. Protein content. The protein content was determined as per
Joharapurkar et al. [25].

Total protein concentration = Abs. of test/Abs. of std × 6.5.

2.4.7. Histopathology of pancreas

On the basis of results, only the control groups and high dose
(200 mg/kg) treated animal groups were shortlisted for the his-
topathological examinations. Isolated pancreas of sacrificed ani-
mals were fixed in 10% formalin solution and immediately
processed by the paraffin technique. Sections of 4 μm thickness
were cut and stained by hematoxylin and eosin (H and E) for
histopathological examination [27]. Well stained sections of
pancreas were studied with the help of Leica digital microscope
(Leica DM 2500, China) with Leica applications suite software.
Pancreatic tissue and islets were observed for their necrosis and/or
regeneration.

3. Results and discussion

3.1. Cultivation

The C. citratus with organic treatment comparative to non-organic
crop showed ten days early initiation of inflorescence. Flowering is an
important step which shows adaptability of plants to seasonal
changes and decides subsequent reproductive success [28]. Floral
development is controlled by both internal and external cues and
elongation of juvenile stage in plants due to any exo and endogenous
factor may be a constraint in production and growth rate of plants
[28]. Out of four morpho-physiological traits, OCC have shown higher
mean values of height (32.66 inches), number of germlasms
(144.30) and root lengths (12.96 inches) [Table 1] while NCC was
found with greater mean values of weight of whole plant (biomass)
(1268.30) [Table 1]. The higher mean values of organic crop may
resulted to the presence of plenty of ‘beneficial soil microbes’ in
organic manure which helps in ‘soil regeneration’ and ‘fertility
improvement’ and protect them from degradation while also pro-
moting growth in plants [29]. The late commencement of adult
vegetative phase might be responsible in non-organic crop for their
low mean values in majority of morpho-physiological traits [30].

3.2. Phytochemical screening

The dried plant materials were extracted with MQ water by tradi-
tional decoction procedure to avoid the incorporation and/or inter-
ference of organic solvents. Surprisingly, the

| Traits of NCC* | Days after transplantation | Organic | Non-Organic |
|---------------|--------------------------|---------|-------------|
|               |                          | mean ± sd | mean ± sd   |
| Preharvest height (inches) | 30 | 19.5 ± 0.52 | 17.91 ± 0.79 |
| 40 | 21.33 ± 0.88 | 20.41 ± 1.24 |
| 70 | 23.66 ± 0.77 | 22.25 ± 0.86 |
| 100 | 29.83 ± 0.38 | 28.91 ± 0.66 |
| Post-harvest Height (inches) | 100 | 32.66 ± 0.44 | 30.66 ± 0.44 |
| Weight (g) | 100 | 1096.99 ± 14.26 | 1268.3 ± 189.54 |
| Number of germlasms | 100 | 144.30 ± 16.63 | 142 ± 13.97 |
| Roots length (inches) | 100 | 12.96 ± 2.34 | 11.58 ± 1.90 |

*No. of replicates was 12.
3.4. Pharmacological profiling

In acute toxicity study, all the extracts were checked to a higher fixed dose of 2000 mg/kg in mice and no lethality or toxic effects were observed. One tenth of the higher fixed dose (200 mg/kg) and another lower dose (100 mg/kg) were selected for the study. Type 2 diabetes mellitus is a major endocrine disorder and a deadly disease in human beings [31]. According to the recent estimations, the prevalence of diabetes in the world would reach to 552 million people in 2030 [32]. STZ and NAD induced type 2 diabetes (non-insulin dependent diabetes mellitus) model was utilized for comparing the hypoglycemic potential of OCC and NCC and the animals with blood glucose ≥ 180 mg/dL were considered diabetic [22]. After induction of diabetes in albino mice, the blood glucose concentrations increased (p < 0.01) compared with normal control group.

3.4.1. Acute anti-diabetic studies

Acute anti-diabetic activity was assessed by blood sampling at two different conditions i.e. fasting (0 and 1.5 h) (preprandial) and postprandial (4 and 6 h) (PPG) [Fig. 3]. The C. citratus have shown the prominent decrease of blood glucose at postprandial condition [Fig. 3]. However, significant decrease in blood sugar found by OCC-200 at 6th h (48.86%) [Table 2]. In type 2 diabetes, peak PPG occurs at about 2 h after a meal and relates to inadequate glucose disposal. The pathogenesis of PPG elevation in type 2 diabetic conditions results from loss of first-phase insulin secretion, failure to control hepatic glucose production and resistance of muscle to glucose uptake. In addition, postprandial hyperglycemia is driven by lack of suppression of glucagon hence, the C. citratus may consider to act through antagonizing the glucagon [33].

3.4.2. Sub-chronic anti-diabetic studies

The organic plant extract exhibited potent anti-diabetic activity comparative to diabetic control and also non-organic plant extract [Fig. 3]. Amongst the extracts, OCC at the dose of 200 mg/kg showed significant (p < 0.01) decrease in blood glucose (166.66 ± 0.94 mg/dL). The order of anti-diabetic activity was observed as positive control > OCC-200 > OCC-100 > NCC-200 > NCC-100 with percent decrease in blood glucose level as 58.56 ≥ 43.44≥32.21 ≥ 29.32≥29.16 respectively [Table 3]. The cultivation standards and time of harvest are the key factors influencing the chemical composition, quality and quantity of the plant constituents including essential oil. With the possibility that the secondary metabolites including polyphenols, flavonoids and saponins may interact with its constituents to potentiate their anti-diabetic effect.

![Fig. 1. Toxicity assay of C. citratus extracts – qualitative analysis: A and B represent the decrease in bioluminescence with naked eye at different concentrations (mg/ml) of OCC and NCC respectively.](Image)

![Fig. 2. Toxicity assay of C. citratus extracts – quantitative analysis: percent luminescence inhibition vs. different concentrations (mg/ml) of OCC and NCC. The results were expressed as mean ± SEM, (n = 3) using analysis of variance (ANOVA), followed by Bonferroni’s multiple comparison test. Where a: p < 0.05 vs. 1, b: p < 0.05 vs. 2, c: p < 0.05 vs. 3, d: p < 0.05 vs. 4, e: p < 0.05 vs. 5, f: p < 0.05 vs. 6, g: p < 0.05 vs. 7, h: p < 0.05 vs. 8 (mg/ml) respective concentrations of OCC and NCC.](Image)
3.4.3. Effect of plant extracts on the body weight of diabetic mice

As demonstrated in the results, induction of diabetes resulted in significant decrease in body weight of diabetic control mice compared with normal control at the end of experiment \( (p < 0.01) \). Administration of extracts (organic and non-organic) to the diabetic mice improved the body weight of animals at the end of 3 weeks which was found significant at the dose of 200 mg/kg \( (p < 0.01) \) compared to the diabetic control group. The order of synergistically; further investigation regarding their interactions would be rewarding \[34\].
result was in the increasing pattern of: OCC-100 < NCC-100 < NCC-200 < OCC-200 < normal control group, in a range of 0.12–9.60%, which might be due to its protective effect in controlling muscle wasting, that is reversal of gluconeogenesis, and might also be due to the improvement in insulin secretion and glycemic control [Table 4][35].

3.4.4. Estimation of lipid profile and liver enzymes in blood serum of diabetic mice

The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia [36]. In our study, after induction of diabetes, a marked elevation in serum TG, TC, VLDL, LDL and reduction of HDL levels were observed in mice.

Table 6

| Treatments | LPO (µM of MDA/g tissue) | SOD (IU/mg protein) | Cat (IU/mg protein) | GSH (IU/mg protein) |
|------------|-------------------------|---------------------|--------------------|---------------------|
| Normal Cont| 0.44 ± 0.03             | 13.82 ± 3.51        | 35.61 ± 2.33**     | 24.45 ± 3.71**      |
| Positive Cont| 0.31 ± 0.02             | 11.47 ± 3.41        | 32.07 ± 2.53**     | 17.2 ± 3.53**       |
| Negative Cont| 1.28 ± 0.03             | 7.42 ± 1.37         | 20.76 ± 1.65ΨΨ     | 5.43 ± 2.06ΨΨ      |
| OCC-200    | 0.78 ± 0.01*            | 12.56 ± 1.34        | 30.78 ± 1.92*      | 14.99 ± 1.95*       |
| NCC-200    | 0.89 ± 0.06             | 9.73 ± 1.036        | 29.21 ± 1.744      | 13.18 ± 1.222       |
| Normal Cont| 0.34 ± 0.009            | 7.54 ± 2.19         | 82.36 ± 6.17**     | 47.68 ± 7.12**      |
| Positive Cont| 0.39 ± 0.01             | 6.01 ± 1.05         | 67.12 ± 6.74**     | 39.48 ± 4.36**      |
| Negative Cont| 1.4 ± 0.02              | 3.79 ± 0.82         | 44.53 ± 3.13ΨΨ     | 21.80 ± 1.97ΨΨ      |
| OCC-200    | 0.73 ± 0.04*            | 4.70 ± 0.32         | 55.87 ± 2.10*      | 28.67 ± 1.74        |
| NCC-200    | 0.95 ± 0.08             | 4.08 ± 0.06         | 45.91 ± 4.75       | 23.64 ± 1.84        |

*p values: **<0.01, *<0.05 compared to negative control group and ΨΨ<0.01, Ψ<0.05 compared to normal control group.

Fig. 5. Hematoxylin and eosin stained histopathological sections of pancreas after 21 days treatment of different test groups. Represents 1) Normal control: Normal architecture (white color) of islet and pancreatic tissue; 2) Negative control: (Diabetic control) Infiltration and degeneration (red color) can be seen in the pancreatic tissue; 3) Positive control: (Glibenclamide group) Enlarged islet (yellow color) trying to meet the demand; 4) OCC-200: Enlarged islets (yellow color) along with different size neo islets (blue color) and showed minor degenerative changes in pancreatic tissue; 5) NCC-200: Islets of Langerhans displaying heavy degenerative (red color) changes compared with normal control group.
compared to normal control [Fig. 4], which are in agreement with Watcho et al. findings. Significant reduction in serum lipid levels in diabetic mice on treatment with the OCC compared with NCC might have caused as a result of insulin levels increment [Fig. 4]. There was increase in hepatic enzymes (SGOT and SGPT) in the serum of diabetic mice compared with normal control (p < 0.01) which might be primarily due to leakage of these enzymes from liver cytosol into bloodstream as a consequence of hepatotoxic effect of STZ. The organic OCC–200 gave the highest (p < 0.01) decrease in the SGOT and SGPT level 140 ± 1 and 100.9 ± 1.29 units/L respectively [Table 5], which showed the protective effect and normal functioning of liver in reversing the organ damage due to diabetes which was clearly observed by high levels of SGOT and SGPT in diabetic control. As liver is an important organ involved in carbohydrate metabolism, the positive effect of the extracts on liver function may be associated with their anti-hyperglycemic characteristic.

3.4.5. Anti-oxidant activity of C. citratus extracts

Hyperglycaemia causes oxidative damage by generation of reactive oxygen species and results in the development of complications. Thus in the present study, the role of extracts in the management of free radicals/ROS were determined in the erythrocyte lysate and liver. As per the previous results, the animals from group A and the higher dose (200 mg/kg) treated animal groups were shortlisted for further analysis. In the current study, the SOD, CAT and GSH activities of diabetic mice erythrocyte lysate and liver were significantly reduced. This may be due to the production of reactive oxygen free radicals that can themselves reduce the activity of these enzymes. The lowered glutathione level in diabetes has been considered an important indicator of increased oxidative stress. Polyphenols have been shown to be potential antioxidants in the treatment of STZ-NAD induced oxidative stress in diabetic animals. The organic crop showed the pronounced increase in SOD, CAT, GSH levels and significantly inhibited the increase in LPO level in E. lysate and liver [Table 6]. It is possible that the delay in STZ-NAD induced oxidative stress in various tissues of C. citratus extract treated mice is predominantly due to its anti-oxidant activity [37].

3.4.6. Histopathology of pancreas

Histopathological examination proved the normal architecture of islet, pancreatic tissue and cellular populations in the normal control group, however, infiltration, necrosis of islet and degeneration in pancreatic tissue was observed in diabetic control. Glibenclamide group was found with enlarged islet to meet the demand of insulin. OCC–200 represented the enlarged and different population in pancreatic tissue which conformed the higher hypoglycemia with higher insulin secretion. NCC–200 displayed heavy degenerative changes and revealed their lower anti-diabetic potential [Fig. 5]. The potent anti-diabetic activity of OCC may therefore be attributed to the higher amount of secondary metabolites.

4. Conclusion

Worldwide revolution for the improvement of people safety is gaining momentum; hence drug safety becomes even more prominent in the present day scenario. Different cultivation practices and laboratory generated production of the medicinal plants or foods are being utilized for commercial purposes. These practices may have profound impact on the safety and efficacy of the Ayurveda drugs in the market. Hence, the present research work attempted to scientifically validate the impacts of organic and conventional cultivation practices on nourishment, biological activity and safety measures. The scientific results proved the organically grown C. citratus is better in terms of nourishment, biological activity and safety measures.

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Conflict of interest

None

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